

EUROGIN 2016 ABSTRACTS

Part I – Main Conference Program

MTC MAIN TRAINING COURSES.....	P.2
STC SATELLITE TRAINING COURSES.....	P.36
MSS MAIN SCIENTIFIC SESSION	P.59
SSIM SCIENTIFIC SESSIONS ON IMMUNOLOGY AND IMMUNOTHERAPEUTICS	P.105
ES EDUCATIONAL SESSION.....	P.134
HN HEAD AND NECK SESSIONS.....	P.136
CS CLINICAL SESSIONS.....	P.200
WACC WACC SESSIONS.....	P.238
SS SCIENTIFIC SESSIONS.....	P.287
GS GERMAN LANGUAGE SESSIONS.....	P.409

MTC 01-01

The burden of HPV associated cancers in men and women

G. Clifford, S. Franceschi, M. Plummer, C. De Martel

IARC (France)

Background / Objectives

The contribution of infections to the global burden of cancer has been assessed periodically, and in the last assessment for 2008, it was estimated that 610 000 (4.8%) of all cancers worldwide were attributable to HPV (de Martel 2012). We have since updated these statistics for the year 2012 using estimates of global cancer incidence from Globocan 2012, as well as improved estimates of population attributable fractions (PAF) for infectious agents derived from a recent literature review, including a new attribution of a small proportion of oral cavity and larynx cancers to HPV.

Methods

The fractions of all cancers attributable to HPV in women and men in 2012 were compared worldwide by 8 geographical regions, and according to the Human Development Index (HDI). Separate estimates are presented for countries that have a large population or a distinct level of economic development compared to other countries in the region (China, India, Japan, South Korea, Australia and New Zealand). The PAF for HPV was estimated to be 100% for cervical cancer, 88% for anal cancer, 78% for vaginal cancer, 51% for penile cancer, 25% for vulvar cancer, 4% of oral cavity and larynx cancer and a variable proportion of oropharynx depending on region (from 15-69% depending on world region).

Results

Of newly diagnosed cancer cases worldwide in 2012, 640,000 were estimated to be attributable to HPV, of which 570,000 were diagnosed in women, and 66,000 in men. These included 270,000, 280,000 and 90,000 cases diagnosed in age groups <50, 50 to 69 years and 70+ years, respectively. Among women, the large majority of the burden was contributed by cervical cancer (530,000),

followed by Anus (18,000), Vagina (12,000), Vulva (8,500), Oropharynx (5,500), Oral cavity (3,000) and Larynx (860) cancer. For men, the contribution was from Oropharynx (24,000), Anus (17,000), Penis (13,000), Oral cavity (5,600) and Larynx (6,400). In low-HDI countries, HPV-related cancers constitute half of all infection related cancers, but the proportion of all infection-related that are caused by HPV decreases with HDI level, mainly due to the screening and treatment of cervical precancerous lesions.

Conclusion

In every world region, the burden of HPV-related cancer is driven by cervical cancer incidence. Differences in the burden of HPV-related cancer between the two sexes in any world region thus depends mainly on: 1) the effectiveness of cervical screening programs; and, to a lesser extent, 2) the fraction of oropharyngeal cancer attributable to HPV.

References

[de Martel C, et al. Lancet Oncol;13(6):607-15 (2012)].

MTC 01-02

The state of the art of HPV epidemiology, cervical vs oral

S. De Sanjose

Catalan Institute of Oncology (Spain)

Background / Objectives

Human Papilloma Virus is one of the most common viral agents infecting skin and mucosae in humans. It is now well established that persistent HPV infection with some specific HPV types, the so called high-risk types, leads to a deregulation of viral gene expression and altered cell functions including cell proliferation, poor DNA repair, and accumulation of genetic changes. All these changes are linked to anogenital cancers including cervix, vagina, vulva, anal canal, penis, and head and neck cancers, particularly oropharyngeal. The HPV aetiological contribution differs in each location reflecting different natural history and different tropism. HPV contributes to over 530,000 new cervical cancer cases and over 80,000 of other related sites every year worldwide. Geographical and social differences in incidence and mortality are prominent particularly for cervical cancer as its burden is highly related to screening and treatment facilities.

Methods

A literature search within Pubmed on HPV and epidemiology of Cervical cancer and HNSCC has been carried out.

Results

Over the last decade, increasing amount of information on the role of HPV in head and neck squamous cell carcinomas (HNSCC) has been amassed. While HPV persistent infection is the necessary cause of the vast majority of cervical cancers this is not the case for cancers of the vulva, penile and HNSCC. Nowadays, it is widely accepted that HPV-positive HNSCC differ significantly from HPV-negative HNSCC, mainly caused by tobacco and alcohol, on the genetical, molecular, epidemiological and clinical level. The HPV involvement in the carcinogenic process may derive in a major impact in the clinical management of HNSCC patients. This is distinctive from the accepted treatment regimes in other HPV related cancers in which HPV involvement has not been yet associated to differential regimes.

Conclusion

Prevention strategies are moving towards incorporating HPV testing in screening practices and HPV vaccination as primary prevention of cervical cancer and other ano-genital cancer. However, the impact of secondary preventive measures in HNSCH HPV positive cases need to be developed and evaluated. It is expected that prophylactic vaccines could have an impact in HNSCC.

MTC 01-04

Natural history: insight into the susceptibility by sites

P. Gravitt

Dept of Global Health, Milken Institute of Public Health, George Washington University (United States of America)

Background / Objectives

HPV genotype tropism and distribution is similar between the cervix and oral cavity. However, the epidemiology of HPV infection in healthy individuals is remarkably different by site, with at least 2-fold higher prevalence in the cervix compared with the oral cavity, even in women with high exposure at both sites (e.g., sex workers). While both oral and cervical HPV infection prevalence estimates are higher in women with HIV-associated immune suppression, the predominance of infection at the cervix relative to the oral cavity remains. HPV infection prevalence at the cervix is lower in older women, whereas oral HPV prevalence shows a bimodal peak at young and older ages. The cause of the prevalence differences in the oral cavity and cervix is not clear. Some current hypotheses to explain this difference include (1) sequence of site-specific exposures where infections occurring first in the genital tract offer protection in distal sites, (2) differences in immune responses by anatomic site, and (3) mechanical or cell-type differences in infection susceptibility in oral vs. cervical epithelium.

MTC 02-01

Primary prevention: Recognizing the respective value of HPV prophylactic vaccines by sites

E. Joura

Medical University Vienna (Austria)

Background / Objectives

Prophylactic HPV Vaccines were originally developed to prevent cervical cancer. Today we know that a wide variety of genital and oropharyngeal cancers are caused by oncogenic HPV. Genital precancerous lesions also cause a substantial burden of disease and enormous cost for public health.

Methods

The attribution of various HPV types in cancers and precancers of the cervix, the vagina, the anus, the penis and the oropharyngeal will be analysed. With the demonstrated efficacy of the currently available prophylactic HPV vaccines for the prevention of precancer at the various anatomic sites, the potential for prevention can be estimated.

Results

HPV 16 is the most dominant oncogenic type in all HPV related cancers. With the first generation of HPV vaccines approximately 70% of HPV related cancers could be prevented, with the second generation (ninevalent HPV vaccine) 90% of invasive cancers and precancers could be prevented in longterm.

Conclusion

Today we have tools available to prevent most HPV related cancers in the future. The challenge is to achieve a good coverage and to distribute the vaccines in low- resource countries where they are most needed. Vaccination at an early age is most effective, gender neutral vaccination is an important improvement of coverage. Vaccination programs will have a substantial impact of cervical cancer screening programs.

MTC 02-02

Current standards and options for HPV cervical cancer screening in non-vaccinated women

N. Wentzensen

**Division of Cancer Epidemiology and Genetics National Cancer Institute -
Bethesda (United States of America)**

Background / Objectives

Cytology-based cervical cancer screening was introduced decades ago and has resulted in substantial reductions in cervical cancer incidence and mortality. The understanding that HPV is a necessary cause of cervical cancer has led to major advances in primary and secondary prevention of cervical cancer. There are now three major alternatives for cervical cancer screening: cytology, HPV testing, and cytology-HPV co-testing. Cytology screening has lower sensitivity compared to HPV and co-testing and needs to be repeated at shorter intervals to achieve good program sensitivity. Conversely, while HPV testing has high sensitivity and allows extending screening intervals, it may double the number of screen-positive women compared to cytology. Thus, HPV screening requires additional triage markers to decide who among the HPV-positives needs to be referred to colposcopy. Two different algorithms have been approved for primary screening in the United States (US) and in the Netherlands. In the US, women testing positive for HPV16/18 are referred to colposcopy, while women positive for other high risk types are triaged with cytology. In the Netherlands, the recommendation is to do cytology triage for all HPV-positive women. Additional algorithms are currently evaluated in different countries and an overview of the state of the art will be presented at the meeting.

Methods

n/a

Conclusion

n/a

MTC 02-03

Screening: Current standards and options for HPV cervical cancer screening in the vaccinated population

K. Canfell

Cancer Research Division, Cancer Council NSW, Sydney, 2011, NSW, Australia (current affiliation) and School of Public Health, University of Sydney, NSW, Australia (Australia)

Background / Objectives

Background: Prophylactic vaccines against HPV have been introduced in most developed countries over the last decade. In a number of settings, vaccine-induced reductions in infections with vaccine-included HPV types and cervical cancer precancerous abnormalities have already been documented in young women. These vaccine-induced changes are helping drive changes to established cervical screening programs in a number of countries.

Objective: To review the options for cervical screening in the era of HPV vaccination and to discuss policy developments in cervical screening in countries where the impact of the vaccine has been rapid.

Methods

A number of options can be considered for cervical screening in vaccinated populations. One major discussion centres on whether it is feasible to assess a woman's vaccination status at the point-of-care for screening and tailor the screening approach accordingly. An alternate approach is to seek a single screening solution that is optimised for both vaccinated and unvaccinated women. Modelling and new data from a major Australian trial ('Compass') are demonstrating the role of primary HPV screening for both unvaccinated and vaccinated women. A number of testing and potential triaging options exist, including co-testing with cytology, primary HPV screening with cytology triage, and primary HPV screening with partial genotyping and referral of HPV 16/18 to colposcopy. Their relative effectiveness and cost-effectiveness will be discussed.

Conclusion

Primary HPV screening with partial genotyping for HPV 16/18 allows the cervical screening test to be tailored to detect and manage the same types that the HPV vaccine protects against. In this way, vaccination and screening programs can be tailored to directly complement each other.

MTC 02-04

OROPHARYNGEAL CANCER SCREENING— STRENGTHS AND WEAKNESSES

A.R. Kreimer

**Division of Cancer Epidemiology and Genetics, National Cancer Institute,
National Institutes of Health (United States of America)**

Background / Objectives

The incidence of HPV-positive oropharyngeal cancer (OPC) is expected to continue to increase in the coming decades in many world regions, as the highly-effective HPV vaccines which were first introduced to 11-12 year olds in mid-2000s are not expected to curtail this trend until at least 2060, when the vaccinated birth cohorts reach the median age of OPC diagnosis (~60 years).

Methods

Research in secondary prevention of OPC continues to be important.

Results

Studies have now documented HPV16E6 is present in blood prior to the diagnosis of OPC and that the antibody response is remarkably stable and strong up to 13 years prior to cancer diagnosis. Yet, while the HPV16 E6 serologic data are promising, the incidence of OPC is much lower than the incidence of other cancers for which screening is currently recommended. Higher-risk subgroups will need to be identified for targeted screening, requiring risk stratification prior to serologic testing. The necessary subsequent steps following HPV16E6 seropositivity, including identification of a histologic precursor lesion, improvements in diagnostics, and establishment of low-harm treatment regimens, must also be established.

Conclusion

Secondary prevention of HPV-driven oropharyngeal will be discussed, along with its strengths and weaknesses.

MTC 02-06

Age- and gender-specific control of HPV-associated cancers: the role of primary prevention, screening, and other interventions

E.L. Franco

McGill University - Montreal (Canada)

Background / Objectives

N/A

Methods

N/A

Conclusion

Prevention and control of HPV-associated diseases, both malignant and benign requires strategies that are tailored to specific age groups and are dependent on gender. Although universal HPV vaccination began in earnest nearly 10 years ago by targeting primarily pre-adolescent and adolescent women only, gender-neutral vaccination policies are gradually being adopted in Western countries. Likewise, adoption of molecular HPV testing as a technology in cervical cancer screening has led to a rethinking of the most appropriate ages to screen and of the screening interval. This session covers the diversity of primary and secondary prevention strategies with a view on future directions for the control of HPV-associated cancers. The Chairs will summarize the state of scientific progress in these areas.

MTC 03 I-03

FEASIBILITY AND COST IMPLICATIONS OF INTRODUCING HPV ASSAYS IN LOW AND MIDDLE INCOME COUNTRIES (LMIC) COMPARED WITH HIGH INCOME COUNTRIES (HIC)

H. Cubie

Global health Academy, University of Edinburgh (United kingdom)

Background / Objectives

There is now little dispute about the value of HPV primary screening to identify women at risk of cervical disease and cancer. This talk will cover the practical issues which influence feasibility and cost of introducing HPV testing in LMIC and which differ from those in HIC.

There is a plethora of HPV assays available commercially mostly designed for HIC. Relatively few HPV assays can be described as 'low resource' whether in terms of kit and equipment costs, trained personnel or compatible laboratory and clinic infrastructures to ensure accurate and speedy transport of samples and results. HPV assays are relatively expensive and tend to use difficult-to-dispose-of 'disposables'. Furthermore, only those assays with a quick turn-around are suited to a 'screen and treat' single visit programme, while high throughput platforms require to be centralised and lead to 'two visit' programmes. These are different issues from those faced in HIC, but continue to influence the feasibility of introducing HPV testing.

In high income countries, the major stumbling block to introduction of HPV primary screening is the speed at which the juggernaut of population-based cytology-based screening programmes can be turned round. In sharp contrast, low and middle income countries have little or no cytology; what does exist is not accessible to the majority of women and theoretically, HPV-based programmes should be easier to introduce. Cost remains an issue and visual inspection with acetic acid (VIA) has been implemented as a low technology deliverable programme in a number of countries. However, unless treatment is available for observed lesions, such screening programmes will achieve little. Furthermore, quality assurance, a major element in the success of cytology based programmes is often not considered when other screening modalities are introduced. QA for HPV assays should reach similar standards to cytology QA, adding a further cost dimension, particularly difficult for LMIC. The introduction of national HPV vaccine programmes, largely in HIC and where there is high coverage, will impact on the type of HPV assays required and lead to dramatic changes in screening programmes. Self-sampling is increasingly considered, as evidence mounts that it is as/almost as effective as physician taken samples. This has implications for public health, staffing and follow-up which exist in both HIC and LMIC. However more self-sampling studies are required in LMIC to

provide robust evidence of the quality of samples which are then submitted and of successful communication of HPV results.

MTC 03 I-04

Practical uses: Lab / pathologists vs onsite outpatient clinic

J. Cuzick

Queen Mary University of London (United kingdom)

Background / Objectives

HPV testing is conventionally performed by doctors or nurses but the sample is sent to an off-site pathology lab for processing and results can take month or longer to be returned to the woman.

Methods

Two tests have been developed to provide a rapid turn-around time. Care HPV (Digene/Hologic) still requires a simple lab facility but the results can be obtained within a day and is aimed primarily at hard to reach rural populations where it is desirable to perform screening and treatment if necessary in the same day, eg by screening in the morning and providing the results and simple treatment such as cryotherapy if necessary in the afternoon. A newer test from Cepheid is assayed in a fully self contained cartridge in a multiplex machine within 90 mins and can be performed directly in the office/building where the test was taken.

Conclusion

This has potential uses both in rural population and more generally even in the developed world, where women can be given their result at the sample visit as their screen, thereby minimizing anxiety in the large portion who will be HPV negative. This advantage needs to be balanced against the likely extra cost of this type of test. It may also be possible to assay urine samples and self-collected cervical samples by this method, opening up new possibilities for providing HPV testing in a screening setting.

MTC 03 I-05

E. Validation methods, quality assurance and transport media

J. Dillner

Karolinska Institutet (Sweden)

Background / Objectives

Primary cervical screening with HPV has now been unambiguously shown to be more effective for cervical cancer prevention than screening with cytology. To ensure that the predicted gains are realized also in the real-life programs, the HPV testing needs to follow similarly stringent quality assurance schemes as other screening methods.

Validation of the methods used needs to be done for every certified laboratory, as the performance of the testing is the product of the assay used and the performance of the laboratory. Clinical validation refers to the sensitivity for detecting specimens with CIN2+ and to the specificity of having a reasonably low proportion of cytology-negative women from the general population testing negative. Analytical validation refers to being able to detect a defined amount of HPV DNA (sensitivity measured in International Units) with low likelihood of reporting a sample as positive for an HPV type that the sample does not contain (specificity), which could e.g. be due to incorrect typing or contamination.

For clinical validation and quality assurance purposes, large HPV laboratories are systematically saving the cervical samples (biobanking) to be able to perform a clinical validation for sensitivity and specificity if a new test is being considered (1,2). Proficiency panels for analytical validation are available via the HPV LabNet (3).

Quality assurance involves e.g. continuous use of positive and negative controls and monitoring the read outs from these as well as laboratory audit. Audit has been standard practise in most cytology laboratories for decades and is based on identification and re-reading of prior smears from women who later turn out to be diagnosed with cervical cancer or carcinoma in situ. Laboratory audit for HPV uses exactly the same design (identifying archival samples from women who later develop cancer or carcinoma in situ) and subjecting these samples to re-testing and extended testing for detection of rare HPV types or variants (4).

Self-sampling is becoming an increasingly popular strategy to reach in particular non-attending women, but significantly increases the cost of the screening program. However, there are now many studies that have used the same self-sampling kits as are being used for self-sampling for Chlamydia testing also for HPV self-sampling. These reagents are both inexpensive, extensively used and have been found to produce equally sensitive results (5).

References

1. Cervical cytology biobanking in Europe. Arbyn M, Van Veen EB, Andersson K, Bogers J, Boulet G, Bergeron C, von Knebel-Doeberitz M, Dillner J. *Int J Biol Markers*. 2010 Jul-Sep;25(3):117-25
2. A complex intervention for workflow enhancement at the Swedish cervical cytology biobank. Perskvist N, Björklund C, Dillner J. *Biopreserv Biobank*. 2014 Feb;12(1):69-73. doi: 10.1089/bio.2013.0
3. Global improvement in genotyping of human papillomavirus DNA: the 2011 HPV LabNet International Proficiency Study. Eklund C, Forslund O, Wallin KL, Dillner J. *J Clin Microbiol*. 2014 Feb;52(2):449-59. doi: 10.1128/JCM.02453-13.
4. Laboratory audit as part of the quality assessment of a primary HPV-screening program. Hortlund M, Sundström K, Lamin H, Hjerpe A, Dillner J. *J Clin Virol*. 2016 Feb;75:33-6. doi: 10.1016/j.jcv.2015.12.007.
5. Evaluation of human papillomavirus DNA detection in samples obtained for routine Chlamydia trachomatis screening. Söderlund-Strand A, Wikström A, Dillner J. *J Clin Virol*. 2015 Mar;64:88-91. doi: 10.1016/j.jcv.2015.01.008

MTC 03 I-06

Molecular Markers and new approaches to stratifying disease risk in cervical screening

E. Paraskevaidis

University of Ioannina (Greece)

Advances in technology and scientific techniques created new horizons for improved understanding of the diseases' processes at a molecular level. In the field of cervical pre-invasive and invasive disease, this allowed an in-depth exploration of the neoplastic mechanisms at a molecular level and led to the development of new test and biomarkers, many of which have become commercially available. With the explosion of new biomarkers targeting the viral DNA detection, the expression of oncoproteins and other cellular processes that promote carcinogenesis in the host, questions on how to best use these in different clinical settings are becoming increasingly difficult to answer. With a continuously evolving evidence-base, the development a clinical decision support system is a current unmet need. This can assist clinicians to use these new technologies to promote prevention, personalise management and improve targeted management.

Since 2010 the Hellenic Cervical Pathology Academic (HeCPA) study group, is working on innovative approaches to exploit advanced mathematical and computing tools for the optimal use of ancillary tests that are available nowadays. More recently, we published a prospective multicentric study of a large patient cohort employed advanced neural networks and artificial intelligence techniques for the development of a Clinical Decision Support Scoring System (DSSS). The system developed had the ability to exploit all the biomarker information in order to accurately predict which women had clinically significant lesions with true oncogenic potential (CIN2 or worse) and give a quantified probability for different histological diagnoses. The results clearly and consistently demonstrated that this DSSS could achieve an optimal balance of increased sensitivity and specificity and minimize the rate of false negative and false positive results.

Improved accuracy and clinical decision support systems has important implications to patients, the health systems and policymakers. If these systems can predict with high accuracy women with or without the disease, they have the potential to significantly improve the management of these populations and the quantified probability for all possible histological diagnoses will be available for effective patient counseling at the primary care setting and in the colposcopy clinics.

MTC 03 I-07

WHICH HPV ASSAYS FULFILL REQUIREMENTS FOR CERVICAL CANCER SCREENING

M. Arbyn¹, C. Meijer², P. Snijders³, J. Berkhof⁴, K. Cuschieri⁵, M. Poljak⁶, I. Heard⁷, J.P. Bogers⁸, C. Depuydt⁹, D. Vanden Broeck⁸, I. Benoy⁸, V. Hutse¹⁰, S. Van Gucht¹⁰, J. Bonde¹¹, T. Gheit¹², M. Tommasino¹², M. Pawlita¹³, D. Geraets¹⁴, W. Quint¹⁴

¹Unit of Cancer Epidemiology & Belgian Cancer Centre, Scientific Institute of Public Health, Brussels (Belgium), ²Department of Pathology, VU University Medical Centre, Amsterdam, (Netherlands), ³Department of Pathology, VU University Medical Centre, Amsterdam (Netherlands), ⁴Department of Clinical Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam (Netherlands), ⁵Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh, Edinburgh, Scotland (United Kingdom), ⁶Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana (Slovenia), ⁷French HPV Reference Laboratory, Institut Pasteur, Paris (France), ⁸Laboratory of Molecular Pathology, Algemeen Medisch Laboratorium, Antwerp & National Reference Centre for HPV, Brussels (Belgium), ⁹Laboratory of Molecular Pathology, Algemeen Medisch Laboratorium, Antwerp (Belgium), ¹⁰National Reference Centre for HPV, Scientific Institute of Public Health, Brussels (Belgium), ¹¹Department of Pathology and Clinical Research Center, Copenhagen University Hospital, Hvidovre (Denmark), ¹²International Agency for Research on Cancer, Lyon (France), ¹³Division Molecular Diagnostics of Oncogenic Infections, Research Program Infection, Inflammation and Cancer, German Cancer Research Center (DKFZ), Heidelberg (Germany), ¹⁴DDL Diagnostic Laboratory, Rijswijk (Netherlands)

Background / Objectives

Several countries are in the process of switching to hrHPV testing for cervical cancer screening. Given the multitude of available tests, validated assays which assure high-quality screening need to be identified.

Methods

A systematic review was conducted to answer the question which hrHPV tests fulfil the criteria defined by an international expert team in 2009, based on reproducibility and relative sensitivity and specificity of the candidate test compared to HC2 or GP5+/6+ PCR-EIA. Results of recent test validations according to the VALGENT-2 protocol (1) were added. A first review published in 2015 (2) was updated.

Results

The cobas 4800 HPV Test, Abbott RealTime High Risk HPV, BD Onclarity and the PapilloCheck HPV test were each consistently validated in two or three studies, whereas the HPV Risk Assay was validated in one study. Other tests which partially fulfil the 2009-guidelines are: Cervista HPV HR Test, GP5+/6+ PCR-LMNX, an in-house E6/E7 RT qPCR, and MALDI-TOF (matrix-assisted laser desorption-ionization time-of-flight). The APTIMA HPV assay targeting E6/E7 mRNA of hrHPV was also fully validated. However, the cross-sectional equivalency criteria of the 2009-guidelines were set up for HPV DNA assays.

The updated review (March 2016) revealed two new studies evaluating Anyplex II HPV HR (Seegene, Seoul, Korea) and Xpert HPV (Cepheid, Sunnyvale, USA), respectively. Both assays showed non-inferior sensitivity and specificity for high-grade cervical intraepithelial neoplasia, compared to HC2 or GP5+/6+ PCR-EIA and demonstrated excellent reproducibility.

Conclusion

Only a limited number of existing HPV assays are clinically validated. VALGENT is an international comprehensive framework for HPV test comparison which allows verification of the clinical accuracy criteria for use in cervical cancer screening. Validation protocols may suffer from selection and ascertainment biases. Continuous monitoring of test performance, updating of the list of validated tests and further international consolidation is needed to assure optimal safety of HPV-based screening programmes. Only clinically validated assays should be used. Two new high-risk HPV assays can be added to the list of tests validated for primary cervical cancer screening.

References

1. Arbyn M, Depuydt C, Benoy I, et al. VALGENT: a protocol for clinical validation of human papillomavirus assays. *J Clin Virol* 2016;76: S14-S21.
2. Arbyn M, Snijders PJ, Meijer CJ, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? *Clin Microbiol Infect* 2015;21: 817-26.

MTC 03 I-08

CYTOLOGIC TRIAGE OF HPV POSITIVE WOMEN

G. Ronco

Centre for cancer prevention (CPO) (Italy)

Results

HPV positive women had cytological triage in 3 randomised controlled trials (RCTs) that had follow up for ≥ 2 rounds of screening and were included in a pooled analysis of invasive cancer incidence. In all these RCTs, HPV-positive women with abnormal cytology (although at different cut-off, ranging from ASC-US+ to HSIL+) were immediately referred to colposcopy while the remaining were invited for HPV re-testing (interval 6-18 months) and referred if still positive (frequently also if cytology become abnormal). This approach can be considered as validated.

In all such studies cytology was interpreted blindly to HPV result. If HPV is used as a stand-alone primary test then the cytology reader will be aware of HPV positivity. In a study nested in the NTCC RCT cytology was interpreted a-posteriori with knowledge of HPV positivity. The relative sensitivity for CIN2+ of having HPV testing and informed cytology \geq ASC-US vs. stand alone cytology \geq ASCUS was 1.58 (95% CI 1.22-2.01) while the relative immediate referral was 0.95 (0.86-1.04). The absolute cross-sectional sensitivity of informed cytology (85.6%; 76.6-92.1) among HPV women was indeed similar to that of p16 over-expression observed in another study nested in NTCC while specificity was better (66% vs. 57%). Data from the Finnish RCT also suggest greater sensitivity of informed vs. blind cytology.

In a first survey of routine activity in Italy (immediate referral of HPV+ women if their informed cytology was ASC-US+ and repeat HPV test after 1 year in the remaining with referral if still positive), the proportion of HPV-positive women judged to have cytology varied strongly between the 10 study centres (range 20.0%-56.9%) so as the proportion of CIN2+ detected immediately or including 1-year HPV re-testing (range 49.0%-94.3%). This suggests strong variability in the criteria of interpretation and in sensitivity of triage cytology. However, immediate referral (because cytology was \geq ASC-US) had limited effect on the overall referral (including HPV repeat) and sensitivity of cytology had no effect on overall sensitivity. This shows that such a protocol is robust to variability in cytology interpretation.

Conclusion

n/a

MTC 03 I-09

OPTIONS FOR TRIAGE OF WOMEN WITH POSITIVE CERVICAL HPV TESTS

W. Kinney

The Permanente Medical Group, Oakland, California, US (United States of America)

Background / Objectives

Review of the options for triage of women found to be HPV positive at cervical screening.

Methods

Review of proposed approaches and preliminary data.

Results

Approaches for triage of HPV positive women may be divided into those which seek additional information from cytologic slide preparations and those which utilize molecular testing independent of intact cells. Slide-based options include improvements in computerized interpretation of standard cytologic preparations, and immunohistochemistry staining of cytology preparations for recognition of p16 and Ki67. Investigation of possible improvements in computer imaging is being actively considered. Preliminary data on p16/Ki67 triage from Kaiser Permanente Northern California indicate that p16/Ki67 dual staining is as sensitive and has better specificity, NPV and PPV than cytology for CIN2+ in 1669 HPV positive women, which could permit a significant reduction in colposcopy.¹

Molecular approaches include risk stratification by more complicated sorting of HPV types or subtypes,² which are associated with significantly different risk. Among the DNA types identified as "high risk" there is a wide variation in risk, informing variations in clinical management. Among the subtypes of HPV 16 identified to date, unpublished data suggests a significant variation in risk and variation in association with squamous versus glandular cancers. DNA methylation at specific sites has been shown to be predictive of CIN3+, and recognition of methylation at CADM1 and MAL sites is associated with dysplasia but not normal cervical tissue on biopsy and can be assessed on cervical scrape specimens.³

Conclusion

Multiple options are possible but not yet commercially available in the U.S. for risk assessment of the HPV positive woman. Increasing specificity of screening in the age of vaccination will be paramount. A test more effective than cervical cytology would permit triage of HPV positive women to risk-appropriate management, facilitate introduction of primary HPV screening, decrease colposcopy, and likely decrease identification and treatment of dysplasia that would not be progressive or persistent. Accomplishing this without decreasing programmatic screening sensitivity and thus cancer prevention would be a significant advance.

References

1. [Wentzensen, JNCI, 2015]
2. [Cullen, Papillomavirus Res, 2015]
3. [van Baars et al, Int J Cancer, 2015]

MTC 03 I-10

THE CHALLENGES ASSOCIATED WITH SCREENING VACCINATED WOMEN

K. Cuschieri¹, K. Pollock², T. Palmer³, K. Kavanagh⁴, R. Bhatia³, R. Cameron², M. Cruickshank⁵

¹Royal Infirmary of Edinburgh (United kingdom), ²Health Protection Scotland (United kingdom), ³University of Edinburgh (United kingdom), ⁴University of Strathclyde (United kingdom), ⁵University of Aberdeen (United kingdom)

Background / Objectives

The reduction of HPV infection and associated lesions as a consequence of national HPV vaccination programmes is now being realised in various countries. As current and proposed cervical screening modalities are largely calibrated to disease levels pre vaccination, their performance will undoubtedly be affected as a consequence of the shifting pattern of infection and disease. The aim of this paper is to outline the challenges in defining optimal screening methods for immunised women.

Methods

Scotland is a country with an organised call-recall cervical screening programme, which has also benefitted from a successful national HPV female vaccination programme since 2008 associated with uptake of over 90% in 12-13 year old girls since its introduction. As Scotland initiates cervical screening aged 20, and incorporated an initial 3 year vaccine “catch up” campaign for girls up to the age of 18, immunised women have been entering the cervical screening programme since 2010. Outputs of the National HPV epidemiology and surveillance programme and associated research will be presented which will provide insight into the performance of cytology and HPV based primary screening in immunised women.

Results

At the population level, a significant reduction in HPV infection and disease is associated with vaccination in Scotland. Screening uptake is higher in women immunised as part of catch up compared to unimmunised women. The prevalence of HPV 16, 18, 31, 33, 45 has decreased significantly, as a consequence of a three dose vaccine schedule although a one dose effect on 16/18 infection was also observed. The predictive value of cytology in immunised women reduces significantly, particularly that of low-grade cytology for CIN2+. While a reduction of HPV 16/18 using clinically validated HPV tests has been observed a proportionate increase of HPV “other” may reduce

the specificity of “HPV first” strategies. Triage of HPV first infection using 16/18 typing in immunised women will have diminishing returns as less than 10% of residual HPV infections will be positive for one of those types.

Conclusion

While vaccinated women appear to be willing to engage in screening the predictive performance of cytology will reduce. While this justifies the introduction of more objective tests, HPV first strategies in immunised women will require robust triage given that residual infections will largely be composed of those known to confer a low risk of significant disease.

MTC 03 II-01

The role of HPV genome sequencing

L. Mirabello¹, Y. Meredith², N. Wentzensen¹, M. Cullen², J. Boland², Y. Kai¹, Z. Chen³, Q. Yang², B. Zhu¹, J. Mitchell², D. Roberson², S. Bass², L. Burdett², Y. Xiao¹, S. Wagner², T. Raine-Bennett⁴, T. Lorey⁴, P.E. Castle⁵, R. Burk⁵, M. Schiffman¹

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, MD (United States of America), ²Cancer Genomics Research Laboratory, Leidos Biomedical Research, Inc., Frederick, MD (United States of America), ³Department of Microbiology, The Chinese University of Hong Kong, Hong Kong (Hong kong), ⁴Regional Laboratory and Women's Health Research Institute, Division of Research, Kaiser Permanente Northern California, Oakland CA (United States of America), ⁵Department of Epidemiology and Population Health, at Albert Einstein College of Medicine, Bronx, NY (United States of America)

Background / Objectives

To permit the large-scale study of HPV genome variability and precancer/cancer, we developed low cost, high-throughput next-generation sequencing (NGS) HPV whole-genome methods. We used our viral whole-genome sequencing assay to investigate HPV16's unique epidemiology and cervical carcinogenicity among 4,626 HPV16-infected women in the NCI-KPNC PaP Cohort and SUCCEED study.

Methods

We have developed a PCR based next-generation sequencing (NGS) assay using the Thermo Fisher Life Sciences' Ion Torrent Proton, custom Ion Ampliseq panels and an analytic pipeline to whole-genome sequence HPV16. We have validated NGS variant calls by comparing with Sanger and Illumina based sequence data. We have further designed a NGS assay to sequence the entire genome of the 13 high-risk HPV types concurrently.

Using European sublineage A1 as reference in this mainly White population, we assessed HPV16 genetic variation (from lineage to SNP level) with worst histologic outcome, including: CIN2 (n=1,284), CIN3 (n=1,395), AIS (n=103), SCC (n=187), adenocarcinoma (n=61), controls (n=1,596, ≤CIN1).

Results

A4 (Asian) sublineage was associated with an increased risk of cancer, specifically adenocarcinoma (OR 9.8, 95% CI 2.0-47.7). The non-European lineage B (African-1) conferred significantly lower risk of CIN3 (OR 0.5, 95% CI 0.3-0.9) while lineage C (African-2) yielded increased risk (OR 2.1, 95%CI 1.1-3.9). D2/D3 sublineages were strongly associated with an increased risk of CIN3+, particularly D2 (OR 7.6, 95% CI 3.0-19.5). D2 had the strongest increased risk of glandular lesions, AIS (OR 29.2, 95% CI 8.9-95.5) and adenocarcinomas (OR 137.3, 95% CI 37.2-506.9). At the SNP level, we have identified 2,679 variable positions, with 67 individual European SNPs and 122 non-European SNPs significantly associated with CIN3+. These data allowed us to determine that controls have a significant burden of rare variants. Deep sequencing has also revealed HPV16 variant lineage co-infections in 24.4% of women. HPV16 variant lineage co-infections were linked to multi-HPV-type infections, and lower CIN3+ risk. We detected several hundred different HPV16 isolates in this population.

Conclusion

NGS HPV genome sequencing has enabled the sequencing of thousands of HPV16-containing specimens from epidemiologic studies for the evaluation of the genetic basis of HPV carcinogenicity. HPV16 actually represents hundreds of co-transmitted viral isolates, providing finer detail for epidemiologic study of viral acquisition and persistence/clearance/re-appearance. Viral genetic variation at the variant lineage and SNP levels strongly influences HPV16 carcinogenicity and histologic outcome and helps explain HPV16's unique properties.

MTC 03 II-03

THE ROLE OF HPV DNA TESTING IN URINE AND SALIVA

S. Franceschi¹, U. Tshomo², M.C. Umulisa³, T. Gheit¹, P.J.F. Snijders⁴, A. Vorsters⁵, M. Tommasino¹, I. Baussano¹, G.M. Clifford¹

¹International Agency for Research on Cancer, Lyon (France), ²Jigme Dorji Wangchuck National Referral Hospital, Department of Obstetrics and Gynaecology, Thimphu (Bhutan), ³Ministry of Health of Rwanda, Kigali (Rwanda), ⁴VU University Medical Center, Department of Pathology, Amsterdam (Netherlands), ⁵University of Antwerp, Vaccine and Infectious Disease Institute, Antwerp (Belgium)

Background / Objectives

Cervical-vaginal cells, collected using conventional smears or liquid-based samples are the gold-standard for the detection of cervical HPV infection and cervical precancer. However, there are circumstances in which other types of samples are more informative or feasible, though as yet less well validated, than cervical samples.

Methods

Our group is working with samples from the cervix, the oral cavity and tonsils, and urine. Urine samples are being used to monitor the effectiveness of nationwide HPV vaccination in Bhutan (start: 2010) and Rwanda (start: 2011) in young women who may have initiated sexual activity but are often reluctant to accept a gynaecological examination for the collection of cervical cells. In 2013-14, we performed two school-based HPV urine surveys; 973 female students were recruited in Bhutan and 912 in Rwanda (1) (median age: 19). Participants self-collected a first-void urine sample using a validated protocol. HPV prevalence was obtained using two PCR assays that differ in sensitivity and type spectrum, namely GP5+/GP6+-PCR and E7-MPG. 92% students in Bhutan and 43% in Rwanda reported to have been vaccinated at age 14-18.

Results

In Rwanda, HPV6/11/16/18 prevalence was lower in vaccinated than in unvaccinated students (prevalence ratio, PR=0.12, 95% confidence interval, CI: 0.03-0.51 by GP5+/GP6+, and 0.45, CI: 0.23-0.90 by E7-MPG). For E7-MPG, cross-protection against 10 high-risk types phylogenetically related to HPV16 or 18 was of borderline significance. In Bhutan, HPV6/11/16/18 prevalence by GP5+/GP6+ was lower in vaccinated than in unvaccinated students but CIs were broad. The Study of natural

history of human Papillomavirus infection and precancerous Lesions In the Tonsils (SPLIT) will include 700 age-stratified individuals who underwent tonsillectomy for benign diseases in selected University Hospitals across France (2). SPLIT's preliminary findings are reported elsewhere in this conference (3).

Conclusion

Our urine surveys highlight progresses in the standardization and accuracy of HPV detection in urine and its usefulness to monitor HPV vaccination. Conversely, the role of HPV testing in urine for cervical screening purposes is still unclear and self-collected cervical samples may provide a more acceptable and less demanding (from a laboratory view point) type of sample. HPV testing in oral cells is also a useful in epidemiological tool but the extent to which it can inform on HPV infection in the tonsils (from which most of HPV-associated cancers of the head and neck arise) is yet unclear.

References

[(1) Franceschi S, Umulisa MC, Tshomo U, Gheit T, Baussano I, Tenet V, Tshokey T, Gatera M, Ngabo F, Van Damme P, Snijders PJF, Tommasino M, Vorsters A and Clifford GM. Urine testing to monitor the impact of HPV vaccination in Bhutan and Rwanda. *Int J Cancer* 2016; submitted.

(2) Franceschi S, Combes JD, Dalstein V, Caudroy S, Clifford GM, Gheit T, Tommasino M, Clavel C, Lacau St Guily J, Birembaut P. Deep brush-based cytology in tonsils resected for benign diseases. *Int J Cancer* 2015;137: 2994-9.

(3) Combes JD, Dalstein V, Clifford GM, Gheit T, Tommasino M, Clavel C, Lacau St Guily J, Franceschi S. Study of HPV and precancerous lesions in the tonsils ("SPILT"): preliminary results. Abstract EUROGIN 2016, June 15-18 2016, Salzburg, Austria.]

MTC 03 II-04

Prospects for Genomics

A.T. Lorincz

Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London UK. (United kingdom)

Background / Objectives

Genomics can mean different things but from a broad perspective it includes a deep and detailed study of DNA and RNA sequences encompassing most or all of the genome using bioinformatics and machine learning computational approaches. The method can generate vast amounts of sequence data from a tissue sample, annotating, aligning, sorting and filtering the data into refined subsets of interest. The genomics approach allows an investigator to find any given biomarker needle in the proverbial genetic haystack repeatedly and with high accuracy. The combination of next generation sequencing (NGS) and bioinformatics pipelines have allowed genomics to revolutionize the study of biological systems. In the near future NGS is also likely to transform routine molecular diagnostics. Key concepts of the methodology and applications of genomics will be presented along with current strengths and limitations.

Methods

Review of published scientific NGS papers and experience from the Wolfson Institute NGS team.

Results

Genomics, underpinned first by microarrays and more recently by NGS is an ideal and comprehensive approach to biomarker discovery and validation. Given an adequately powered high-depth NGS experiment a comprehensive dataset can be produced which is an excellent resource for data mining and a reference for the future. Publicly shared NGS datasets can be mined repeatedly with more and more refined computational methods, each time potentially yielding new information. There are some limitations to genomics studies, mostly related to high costs and data overload, these are temporary barriers that will disappear with newer platforms and more competition.

Conclusion

NGS can produce vast amounts of detailed nucleic acid information on biological samples. Bioinformatics pipelines, although cumbersome today, are evolving into more user-friendly formats and will soon become relatively easy to use for non-experts, which will pave the way for genomics to enter into routine diagnostic use.

MTC 03 II-05

THE EXPANDING ROLE OF SELF-COLLECTION

D. Heideman¹, P.S.T. ProhTECT/improve Study Team*²

¹Department of Pathology, VU University Medical Center, Amsterdam (Netherlands), ^{2*} PROHTECT/IMPROVE study team: J Berkhof, FJ van Kemenade, LF Massuger, NJ Polman, VM Verhoef, AT Hesselink, RD Steenbergen, PJ Snijders, CJ Meijer, M Gök, L Rozendaal, R. Ebisch, RP Bosgraaf, RL Bekkers, WJ Melchers, J Bulten, LI Overbeek, AL de Vries, M Babovic, JW Spruyt, F Voorhorst, JA Beliën, (Netherlands)

Background / Objectives

Studies have shown that offering self-sampling for HPV testing (HPV self-sampling) can improve the cervical cancer screening program. In two studies in the Netherlands (PROHTECT-1 and -2), in which HPV self-sampling was offered to non-attendees of the regular screening programme, about 30% of women responded by submitting a self-sample. Pooled data from these studies show that HPV self-sampling targets a substantial portion of non-attendees of all ethnic groups who have not regularly been screened. The data indicate that HPV self-sampling can reach the women at highest risk of cervical cancer.

Methods

In a third study (PROHTECT-3), a molecular triage assay directly applicable to self-samples of HPV-positive women was evaluated against cytology triage on a physician-taken follow-up cervical scrape. Molecular triage (i.e., DNA methylation analysis of cancer-related genes) on HPV-positive self-samples was found non-inferior to cytology triage in the detection of CIN2+. This molecular approach obviates the need for an additional visit to a physician and reduces time to CIN2+ diagnosis, but at the cost of more colposcopy referrals. In another study (PROHTECT-3b), the performance of two self-sampling methods (brush and lavage) was compared among 30,130 women not attending cervical screening. The study showed that offering a brush-based device to non-attendees is non-inferior to offering a lavage-based device in terms of participation. The two self-sampling methods were equally effective in detecting HPV and CIN2+/CIN3+, and performed similarly with respect to user comfort and women acceptance.

Results

In a current study (IMPROVE), a prospective randomized pilot implementation trial of HPV self-sampling in primary screening, a comparison of cervical screening (using HPV testing plus reflex

cytology triage testing) via self-sampling and clinician-sampling among regular screening responders is ongoing. Studies have reported that HPV self-sampling can have a similar sensitivity for CIN2+ as HPV testing on a cervical scrape obtained by a physician, although this may depend on the self-sampling device, HPV testing method and protocols used.

Conclusion

When proven clinically non-inferior to HPV testing on physician-collected cervical scrapes, HPV self-sampling may not only be used to complement current screening programs by increasing screening coverage (i.e., targeting non-attendees), but may also be offered as alternative to all women invited for cervical screening.

MTC 03 II-06

HPV carriage: the urgent needs for education of physicians and public

H. Trottier

Université de Montréal / Sainte-Justine Hospital (Canada)

Background / Objectives

Human papillomaviruses (HPVs) are recognized unequivocally as the main causal factor of all cervical cancers, of a substantial proportion of many other anogenital neoplasms (anal, vaginal, vulvar, and penile cancers), of a non-negligible portion of head and neck cancers (oral cavity, pharynx, and larynx) and is suspected to play a role in other neoplasms as well, such as conjunctiva carcinoma. HPVs also cause benign lesions such as papillomatosis and condylomas. HPVs are also the most common sexually transmitted infections in the world. About all sexually active individuals will be infected with HPV at least once in their lives.

Methods

Importantly, most HPV infections are asymptomatic and only a small proportion of infected individuals will progress to persistent infection and ultimately toward cancer, as most people will clear the infection within 12-24 months. This means that in most people, HPV will just be eliminated without any clinical consequences. In some cohorts of young individuals, the prevalence of HPV can reach 40-60%. It is important to keep this matter in proportion and to avoid crying wolf with alarmist statements following the detection of HPV. It might be very stressful for patients to have the announcement that their HPV test was positive. They can worry about the possible clinical consequences and maybe even more about the aspect of infidelity of their partner.

Conclusion

Education is needed for physician and public to put everything in perspective.

MTC 03 II-07

Conclusion: which test for which circumstances: countries, target users, population, sites

P. Gravitt

Dept of Global Health (United States of America)

The revolution in molecular diagnostics has led to the development of a marvelous array of options for the prevention and management of HPV-associated diseases. The evidence base for any individual test or strategy varies, but is generally quite strong, moving the conversation increasingly away from strict ranking of test performance to discussions of which test is best able to meet the needs and resources of the situation. This talk will discuss the opportunities these technologies provide to locally adapted and appropriate HPV-associated disease prevention. The revolution in molecular diagnostics has led to the development of a marvelous array of options for the prevention and management of HPV-associated diseases. The evidence base for any individual test or strategy varies, but is generally quite strong, moving the conversation increasingly away from strict ranking of test performance to discussions of which test is best able to meet the needs and resources of the situation. This talk will discuss the opportunities these technologies provide to locally adapted and appropriate HPV-associated disease prevention.

STC 01 A-01

The new ISSVD and consensus terminologies of Vulvar Squamous Intraepithelial Lesion (VSIL) and Vulvodynia

J. Bornstein

Galilee Medical Center and Bar-Ilan University. Chairman ISSVD terminology Committee (Israel)

Background / Objectives

VSIL terminology: The introduction of the LAST raised two concerns in relation to vulvar lesions: the absence of reference to "differentiated VIN". Secondly, including the term: 'low grade squamous intraepithelial lesion' (LSIL) in LAST recreated the potential for over-diagnosis.

Vulvar pain and Vulvodynia: New research led to better understanding of the physiology and pathogenesis.

Methods

VSIL terminology: The terminology committee of the ISSVD discussed several new terminology options.

Vulvar pain and Vulvodynia: The ISSVD, together with ISSWSH and IPPS held a consensus conference.

Results

VSIL terminology:

- Vulvar LSIL, encompassing flat condyloma or HPV effect
- Vulvar HSIL (VIN usual type)
- VIN, differentiated-type (DVIN)

2015 Consensus terminology and classification of persistent vulvar pain and vulvodynia

A. Vulvar pain caused by a specific disorder*

- Infectious
- Inflammatory
- Neoplastic
- Neurologic
- Trauma

- Iatrogenic
- Hormonal deficiencies

B. Vulvodynia – Vulvar pain of at least 3 months' duration, without clear identifiable cause, which may have potential associated factors

Descriptors:

- Localized or Generalized or Mixed (localized and generalized)
- Provoked or Spontaneous or Mixed (provoked and spontaneous)
- Onset (primary or secondary)
- Temporal pattern (intermittent, persistent, constant, immediate, delayed)

 *Women may have both a specific disorder (e.g. lichen sclerosus) and vulvodynia

2015 Consensus terminology and classification of persistent vulvar pain and vulvodynia

Appendix: Potential factors associated with Vulvodynia*

- Co-morbidities and other pain syndromes [Level of evidence 2]
- Genetics [Level of evidence 2]
- Hormonal factors (e.g. pharmacologically induced) [Level of evidence 2]
- Inflammation [Level of evidence 2]
- Musculoskeletal [Level of evidence 2]
- Neurologic mechanisms:
 - Central (spine, brain) [Level of evidence 2]
 - Peripheral – Neuroproliferation [Level of evidence 2]
- Psychosocial factors [Level of evidence 2]
- Structural defects [Level of evidence 3]

Conclusion

VSIL terminology: The new terminology includes all types of vulvar squamous intraepithelial lesions and create unity among clinicians and pathologists.

Vulvar pain and Vulvodynia: The new terminology incorporates new information derived from evidence-based studies conducted since the last terminology introduced in 2003.

STC 01 A-02

Efficacy for Vulvovaginal Disease of the 9-valent HPV Vaccine in 16-26 year old women

E. Joura¹, S. Garland², A. Giuliano³, O. Bautista⁴, A. Luxembourg⁴

¹Medical University Vienna (Austria), ²University of Melbourne (Australia), ³Moffitt Cancer Center Tampa, FL (United States of America), ⁴Merck & Co., Inc., Whitehouse Station, NJ (United States of America)

Background / Objectives

An efficacy and immunogenicity study of an investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine was conducted in women 16-26 years of age to demonstrate immunological non-inferiority of HPV 6/11/16/18 response and efficacy against HPV 31/33/4/52/58-related disease. The report presents efficacy against vulva-vaginal disease through end-of-study (i.e. up to month 54 visit).

Methods

14,204 healthy 16-26 year-old women were enrolled into an international, double-blind efficacy and immunogenicity study of the 9vHPV vaccine. Subjects received 9vHPV vaccine or quadrivalent HPV (qHPV) vaccine as a series of injections at day 1/month 2/month 6. Primary analyses included subjects who were seronegative at day 1 and PCR negative from day 1 through month 7 for the HPV type being analyzed. Gynecological examinations were performed every 6 months, and abnormal areas were biopsied.

Results

12,021 women were eligible for this analysis. Efficacy against vulvovaginal disease caused by HPV 6/11/16/18 was equal to qHPV vaccine. Efficacy against HPV 31/33/45/52/58-related VIN/VaIN (any grade) in the primary analysis was 94.4% (95% CI: 67.7, 99.7). No case of high-grade vulvovaginal disease related to the 5 new types was observed in the 9vHPV vaccine group and 3 cases were observed in the qHPV vaccine group. The number of external genital biopsies related to HPV 31/33/45/52/58 was reduced by 92.3% (95% CI: 72.4-98.7).

Conclusion

The 9vHPV vaccine was highly efficacious in preventing HPV 31/33/45/52/58-related vulvovaginal disease up to month 54 visit. Efficacy against disease caused by HPV 6/11/16/18 was the same as with the qHPV vaccine.

STC 01 A-03

Should we administer the HPV vaccine in patients with HPV?

M. Steben

, Clinique A rue McGill - Montreal (Canada)

Background / Objectives

Solid data from the phase 3 studies confirms that the quadrivalent HPV (4HPV) vaccine prevents high-grade disease of the vulva. Solid data from phase 3 study confirms that the protection would be also effective in women already exposed to HPV and would increase in time after vaccination. In the same studies, VIN number was reduced from an incidence of 105 per 10,000 per year in HPV 6-11-16-18 unexposed group to 80 per 10,000 per year in the exposed group and biopsy number was reduced from 130 per 10,000 per year in HPV 6-11-16-18 unexposed to 105 per 10,000 in exposed group.

Post-hoc analysis data presents solid evidence that protection of recurrence of any genital warts, VIN or VAIN was decreased by 44% (95% CI 14-64) in recipients of the 4HPV vaccine compared to a decrease of 79% HPV 6-11-16-18 related genital warts, VIN or VAIN.

In the 3 studies of the phase 3, 4HPV prophylactic vaccine efficacy in women previously exposed to vaccine-related HPV6-11-16-18 whose infection has cleared, seropositive and DNA negative, the incidence rate for external genital lesions, genital warts as well as VIN and VAIN, was 100% (95% CI: 40-100).

In the 9valent HPV (9HPV) prophylactic vaccine protection provided against external biopsy for 9HPV compared to 4HPV vaccine for HPV 31-33-45-52-58 was 90.9% (95% CI 65.7, 98.5) and protection against VIN1+ and VAIN1+ was 91.7%, (95%CI: 51.3, 99.6), but we do not know yet if the protection for HPV 6-11-16-18-31-33-45-52-58, exposed women whose infection has cleared will be as good as for the 4HPV vaccine?

Conclusion

HPV prophylactic vaccines have a place to prevent actual VIN. 4HPV vaccine has been found to have a great role in reducing lesions as well as medical intervention in both unexposed and exposed women. The future will tell us if the same kind of protection against lesions and medical intervention was seen in exposed women receiving the 9HPV vaccine. In the meantime, it is important to know that even if a woman was exposed to one HPV type, she can still benefit from the protection of the extra types included in the 9HPV vaccine.

STC 01 A-04

Multicentric lower genital tract SIL

E. Schejter

Maccabi HMO (Israel)

Background / Objectives

Presentation at the precongress course: vulvar disease A, presided by prof J. Borenstein

The female genital tract, a continuum of squamous epithelium from the vulva to the cervix, is commonly infected by human papillomavirus. The outcome of HPV infection depends on the immune response, the viral genotype (low-risk or high-risk/oncogenic) and the site of infection (the cervical and anal squamo-columnar junction is more susceptible to HPV disease). The key role of HPV in most cancers of the female lower genital tract has been firmly established biologically and epidemiologically

Methods

Review: High-risk human papillomavirus infection usually is seen at one anatomic site in an individual. Rarely, infection at multiple anatomic sites of the female lower genital tract in the same individual is encountered either simultaneously and/or at a later date. Multisubtype infection and infection by rare hrHPV subtypes are common in multifocal dysplasia involving multiple anatomic sites of the female lower genital tract

Results

Women with a history of grade 3 CIN had increased risks of cancer of the vagina (6.74), vulva (2.22), and anus (4.68). Among women with a history of cervical cancer or grade 3 CIN, the incidence rates of anal cancer ranged from 0.8 to 63.8 per 100,000 person-years, and in the general population, the incidence rates ranged from 0.55 to 2.4 per 100,000 person-years.

Conclusion

Squamous cell carcinomas (SCCs) of the vulva develop through human papilloma virus (HPV)-associated or HPV-independent pathways, but the relationship between pathogenesis, classification, and prognosis of these tumors is controversial. HPV-associated neoplasms present in younger women and are associated with usual vulvar intraepithelial neoplasia (VIN), distinct histologic subtypes, and diffuse expression of the cell cycle protein p16 (INK4a). In contrast, HPV-independent vulvar SCCs occur in older women and harbor frequent TP53 somatic mutations. Histologically, non-HPV-associated vulvar SCCs have been associated with differentiated VIN and lichen sclerosis

References

disease. *J Pathol.* 2015 Mar;235(4):527-38.

Mario Preti et al, VIN usual type—from the past to the future, *ecancer* 2015, 9:531

Zekan J, Skerlev M, Milić L, Karelović D. Human papillomavirus-related diseases of the female lower genital tract: oncogenic aspects and molecular interaction. *Coll Antropol.* 2014 Jun;38(2):779-86

Edgren G, Sparén P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. *Lancet Oncol.* 2007 Apr;8(4):311-6

Hui Y, Manna P, Ou JJ, Kerley S, Zhang C, Sung CJ, Lawrence WD, Quddus MR. High-risk human papillomavirus infection involving multiple anatomic sites of the female lower genital tract: a multiplex real-time polymerase chain reaction-based study. *Hum Pathol.* 2015 Sep;46(9):1376-81

STC 01 A-05

Treatment of VLSIL and early invasive vulvar cancer.

M. Roy

Laval University (Canada)

The treatment of VLSIL and early invasive vulvar cancer are now conservative. Total simple vulvectomy has been replaced by partial vulvectomy (wide local excision), laser vaporization, and immune response modifiers (IRMs), Imiquimod. Because VLSIL is found more often in younger patients, even "conservative treatment" can be too aggressive. For example, extensive laser vaporization can cause important pain, excessive healing time and scarring of the tissue. Biopsies are mandatory when VLSIL is suspected to make sure that early invasive cancer present. When it happens, conservative treatments are local excision alone in stage 1a, and in stage 1b, local excision with sentinel nodes mapping are the best choice when the SN are negative.

STC 01 A-06

VULVAR CANCER – DIAGNOSIS AND MODERN TREATMENT

M. Preti¹, L. Micheletti¹, J. Bornstein²

¹DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF TURIN (Italy), ²DEPARTMENT OF OBSTETRICS & GYNECOLOGY, GALILEE MEDICAL CENTER - NAHARIYA (Israel)

Background / Objectives

Vulvar cancer is a rare disease with a bimodal age distribution. Risk factors include HPV infection in younger women and lichen sclerosus and planus in older women. The 2015 ISSVD terminology encompasses Vulvar High-grade Squamous Intraepithelial Lesions and Vulvar Intraepithelial Neoplasia, differentiated type underlining the two different etiologies of vulvar cancer.

Methods

Review of current scientific literature

Results

No systematic screening is available and recognition of precursors and invasive vulvar cancer relies on the presence of vulvar symptoms and on the accuracy of healthcare provider inspecting the vulva. Clinical examination must include direct extension of vulvar cancer to adjacent structures (urethra, vagina and anus) and fixation to the bone. Preoperative lymphnode evaluation with positron emission tomography (PET) scan may be helpful in selected cases.

Stage of vulvar cancer defines both treatment options and prognosis. Vulvar cancer is staged surgically with depth of stromal invasion and involvement of inguino-femoral nodes being the most important prognostic factors.

Conservative surgical techniques are evolved both for vulvar and nodal surgery with the aim of minimizing morbidity maintaining disease free and overall survival similar to more extensive surgical approach.

With this intent surgical treatment of vulvar lesion shifted during last decades from “en bloc” cancer and inguinal nodes dissection toward local radical resection, defined as a surgical excision of the

lesion with at least 1 cm free deep and lateral margins, and separated inguinal incisions for node dissection.

Appropriate surgical assessment of the inguinal lymph nodes is imperative because groin node recurrences are almost all fatal. In unifocal lesion, less than 4 cm and without clinically suspicious groin node a sentinel node biopsy may be, if negative, a less morbidity alternative to complete inguino-femoral node dissection. Only centers with appropriate surgical, nuclear medicine and pathological expertise are candidate to perform sentinel node biopsy as omission of involved nodes identification leads patients to lethal groin progression of cancer.

Adjuvant groin and pelvic radiation and in selected studies chemotherapy in addition to radiation depends on type, number and side of involved inguinal nodes.

Tailored neoadjuvant chemotherapy and radiation is used to treat advanced vulvar cancer to preserve anal, rectal and bladder function.

Treatment of recurrent disease is determined by the localization of recurrence and prior treatment.

Conclusion

Management of vulvar cancer should be individualized and requires an experienced, multidisciplinary team approach in an oncological center.

STC 01 B-01

Inflammatory Aspects in the Etiopathogenesis of Vulvodynia

P. Tommola

Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (Finland)

Localized provoked vulvodynia (LPV) presents with induced pain by touch on vulvar mucosa in the absence of any other recognizable disease (1). Vestibulodynia represents the pain sensation in the vulvar vestibular mucosa and results in severe dyspareunia. Pain intensity is out of proportion to the applied pressure: very light touch evokes excessively strong pain (allodynia).

Opinions on the etiopathogenesis of LPV differ, and the mechanisms resulting in the altered pain sensation are unknown. Recurrent vulvovaginal candidiasis has been considered as a risk factor for the disease (2). A tendency to an exaggerated inflammatory response and dysregulation of inflammation in affected women has been suggested (3). Also, there is evidence for a special genetic characteristic associated with an increased risk of recurrent vulvovaginal candidiasis in women with LPV (4). Since the immune and neuronal systems are closely interrelated an increased immunoinflammatory response may well predispose to the development of a chronic pain syndrome. The vulvar vestibule has also been suggested to possess a unique, embryologically defined immunoinflammatory responsiveness. In a recent study fibroblasts derived from a painful vestibular area were capable of producing a far more extensive inflammatory response than fibroblasts originating from other or non-painful areas of the vulvar mucosa (5).

Recently we demonstrated the existence of secondary lymphoid tissue, the vestibule-associated lymphoid tissue (VALT) in the vestibular mucosa and showed that VALT had become activated in LPV (6). In VALT, like in other mucosa-associated lymphoid tissues (MALT), the initiation of an immune response (B and T cell activation) takes place in germinal centers. In our material of 27 LPV patients and 15 healthy controls germinal centers were present in 14 patients as a sign of immune activation, and in none of the controls. We also found more intra epithelial nerve fibers (IENF) in the patients than in the controls (small unmyelinated C-fibers and thinly myelinated A-delta fibers). The IENFs tended to center around areas with increased B lymphocyte infiltration. Also, the density and presence of IENFs were higher in samples with more pronounced immune activation (Tommola P, et.al. unpublished). This further supports the pivotal role of immune activation in the altered pain sensation of LPV. An interplay between activated immune cells and biomodulators of the signaling of sensory neurons could thus be involved in LPV.

References

1. Moyal-Barracco M, Lynch PJ. 2003 ISSVD terminology and classification of vulvodynia: A historical perspective. *J Reprod Med.* 2004;49(10):772-777.

2. Farmer MA, Taylor AM, Bailey AL, et al. Repeated vulvovaginal fungal infections cause persistent pain in a mouse model of vulvodynia. *Sci Transl Med.* 2011;3(101):101ra91.
3. Foster DC, Sazenski TM, Stodgell CJ. Impact of genetic variation in interleukin-1 receptor antagonist and melanocortin-1 receptor genes on vulvar vestibulitis syndrome. *J Reprod Med.* 2004;49(7):503-509.
4. Lev-Sagie A, Prus D, Linhares IM, Lavy Y, Ledger WJ, Witkin SS. Polymorphism in a gene coding for the inflammasome component NALP3 and recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol.* 2009;200(3):303.e1-303.e6.
5. Foster DC, Falsetta ML, Woeller CF, et al. Site-specific mesenchymal control of inflammatory pain to yeast challenge in vulvodynia-afflicted and pain-free women. *Pain.* 2015;156(3):386-396.
6. Tommola P, Butzow R, Unkila-Kallio L, Paavonen J, Meri S. Activation of vestibule-associated lymphoid tissue in localized provoked vulvodynia. *Am J Obstet Gynecol.* 2015;212(4):476.e1-476.e8.

STC 01 B-02

Etiopathogenesis of Provoked vestibulodynia: Genetic aspects

N. Bohm-Starke, U. Hedding

Karolinska Institutet Danderyd Hospital, Stockholm (Sweden)

Background / Objectives

A familial aggregation of PVD has not yet been proven, however twin studies have shown a heritability of 13-50% in other longstanding, predominately female pain disorders, such as fibromyalgia, IBS and migraine. It is thought that a triggering insult is required for a chronic pain condition to develop, but there are also susceptibility factors that might be inherited.

Methods

Candidate gene association studies.

Results

The assumed genetic predisposition for developing PVD has been investigated to some extent. There are scientific evidences of a neurogenic inflammation in the vestibular mucosa in PVD patients and they are more likely to be homozygous for allele 2 of the IL-1 receptor antagonist gene involved in the pro-inflammatory tissue response.

Recurrent vulvo-vaginal Candida infections have been reported as a trigger of PVD symptoms. A higher frequency of a variant of the gene coding for mannose-binding lectin, an innate immune antimicrobial protein that inhibits Candida proliferation, has been associated with PVD.

Also the influence of hormonal contraceptives as risk factor for developing PVD has been targeted genetically. Women carrying a variant androgen receptor gene, with longer CAG-repeats, might be at higher risk of developing PVD while using combined hormonal contraceptives than women carrying shorter alleles.

Some genes effecting endogenous pain modulation has been studied in this patient group. Both the opioid and serotonergic systems have wide-ranging actions throughout the body, not limited to pain modulation but also effects anxiety, stress response, sexual behavior and sexual function. The A118G SNP in the OPRM1 gene results in an altered μ -opioid receptor with a supposedly higher binding affinity of β -endorphin. The 118G-haplotype has been associated with pain protection, lower PPTs and less chronic pain. The 118A genotype of the the OPRM1 gene has been found to be more common in PVD patients than in controls.

The A-1438G and T102C SNPs in the 5HT-2A gene has been associated to an altered function of the serotonin receptor. This polymorphism has been associated to fibromyalgia, TMD and CWP. The TC/CC genotype of the 5HT-2A gene was more common in PVD patients than in controls, strengthening the notion of PVD being a disorder akin to these.

Conclusion

Differences in inflammatory response and immune defense might explain pain developing after candida infection.

Differences in androgen receptor function and GCH1-gene polymorphism might explain pain developing after use of hormonal contraceptives.

Differences in the opioid and the serotonergic systems might contribute to both pain hypersensitivity and psychosexual characteristics seen in this patient group.

References

1. Witkin, S.S., S. Gerber, and W.J. Ledger, Influence of interleukin-1 receptor antagonist gene polymorphism on disease. *Clin Infect Dis*, 2002. 34(2): p. 204-9.
2. Jeremias, J., W.J. Ledger, and S.S. Witkin, Interleukin 1 receptor antagonist gene polymorphism in women with vulvar vestibulitis. *Am J Obstet Gynecol*, 2000. 182(2): p. 2835.
3. Foster, D.C., T.M. Sazenski, and C.J. Stodgell, Impact of genetic variation in interleukin-1 receptor antagonist and melanocortin-1 receptor genes on vulvar vestibulitis syndrome. *J Reprod Med*, 2004. 49(7): p. 503-9.
4. Foster, D.C. and J.D. Hasday, Elevated tissue levels of interleukin-1 beta and tumor necrosis factor-alpha in vulvar vestibulitis. *Obstet Gynecol*, 1997. 89(2): p. 291-6.
5. Gerber, S., et al., Interleukin-1beta gene polymorphism in women with vulvar vestibulitis syndrome. *Eur J Obstet Gynecol Reprod Biol*, 2003. 107(1): p. 74-7.
6. Babula, O., et al., Immunoglobulin E antibodies to seminal fluid in women with vulvar vestibulitis syndrome: relation to onset and timing of symptoms. *Am J Obstet Gynecol*, 2004. 190(3): p. 663-7.
7. Babula, O., et al., Altered distribution of mannose-binding lectin alleles at exon I codon 54 in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol*, 2004. 191(3): p. 762-6.

8. Donders, G.G., et al., Mannose-binding lectin gene polymorphism and resistance to therapy in women with recurrent vulvovaginal candidiasis. *Bjog*, 2008. 115(10): p. 1225-31.
9. Lev-Sagie, A., et al., Polymorphism in a gene coding for the inflammasome component NALP3 and recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol*, 2009. 200(3): p. 303 e1-6.
10. Goldstein, A.T., et al., Polymorphisms of the androgen receptor gene and hormonal contraceptive induced provoked vestibulodynia. *J Sex Med*, 2014. 11(11): p. 2764-71.
11. Hedding, U., et al., GCH1-polymorphism and pain sensitivity among women with provoked vestibulodynia. *Mol Pain*, 2012. 8: p. 68.
12. Hedding, U., et al., A118G polymorphism in the μ -opioid receptor gene and levels of β endorphin are associated with provoked vestibulodynia and pressure pain sensitivity. *Scan J Pain* 2013.10.004.
13. Hedding, U., et al. Serotonin receptor gene (5HT-2A) polymorphism and anxiety are associated to provoked vestibulodynia and co-morbid symptoms of pain. *J Sex Med*. 2014;12:3064-71.

STC 02 I-01

DISPARITIES IN THE CERVICAL CANCER BURDEN

A. Anttila

Finnish Cancer Registry, Helsinki (Finland)

Background / Objectives

In the current 28 Member States of the European Union (EU), approximately 34,000 new cases of cervical cancer and 13,500 deaths from the disease occur annually. Despite significant progress, incidence and mortality rates are still high in many of the Member States and there are countries with no clear decrease in cervical cancer burden even if screening practised in large scale. The current variation in cervical cancer burden largely reflects inadequate implementation of cervical cancer screening programmes.

Methods

The European quality assurance guidelines recommend systematic linkages between screening, cancer registry and mortality records. When screening data can be linked with the cancer incidence and mortality records, a comprehensive audit of the entire screening programme can be performed. The presentation discusses key entities in these audits in relation with screening outcome (1).

Results

Each case of cancer should be investigated, i.e., cancers in both screened and unscreened women. Key areas of potential errors in screening affecting cancer burden are

- Whether the target age defined and women invited properly and with appropriate intervals
- If women remain unscreened or underscreened – even though a large proportion of the population may be screened too frequently
- Whether sampling or diagnostic errors in screening test, triage or confirmation
- Were there management errors in pre-cancer cases; e.g. drop-out prior to management or in the management follow-up after the primary excision treatment
- If not optimal treatment of cancer.

Registry-based audit should be carried out for any technologies that are implemented in the programme. In attended women, re-review of negative screening tests of subsequent cases, seeded in a relevant set of controls allow further distinction between reasons for potential false negative diagnoses simultaneously with information on the specificity in re-reading.

Conclusion

An important element of the audit is to monitor the programme policies and service providers over the long term, to demonstrate whether the quality assurance activity contributed to any additional improvement in effectiveness.

References

(1) Anttila A, Ronco G, Lynge E, et al. Epidemiological guidelines for quality assurance in cervical cancer screening. In: Arbyn M, Anttila A, Jordan J, et al., eds. European guidelines for quality assurance on cervical cancer screening. 2 ed. Brussels: European Community, February 2008.

STC 02 I-05

PRACTICAL EXAMPLE OF A CERVICAL SCREENING PROGRAM AUDIT

M. Elfström¹, B. Andrae², J. Dillner³, P. Sparen⁴

¹Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Regional Cancer Center of Stockholm-Gotland (Sweden), ²Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm; Center for Research and Development, Uppsala University/Region of Gävleborg (Sweden), ³Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden), ⁴Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Real-life effectiveness of screening programs can be very different from the theoretical gains. Nationwide case-control audits with standard SOPs and inclusion of HPV data can be used for repeat evaluations over time, including evaluation of whether implemented improvements work as expected and provide evidence for incremental improvements of programs and prioritization of quality assurance efforts. Whether cases occurred because current guidelines have not been followed, can be studied using a case series approach. But to study the effect of the different elements in the screening process, and whether there is need for new recommendations and guidelines, the analyses needed require linkage to registers and comparison with control subjects from the population and should be performed in a research setting using validated data.

Methods

A worked example from the on-going audit conducted by the research arm of the Swedish National Cervical Screening Registry will be presented. Questions addressed by the audit are designed to be relevant and helpful for the responsible actors at the different levels of the screening program. Each aspect of the screening program is examined with data collected in the screening program and healthcare system: optimization of participation, testing quality, screening test methods, triage, referrals, assessment, treatment, and follow up. Issues regarding validity of the data should also be addressed.

Conclusion

The Swedish audit procedures have been developed over two rounds of data collection and analysis. The process requires significant investment but is based on routinely reported data and the analyses have led to concrete results that can be used for programmatic changes. In Sweden, the standardized audit protocol will be repeated regularly to monitor effectiveness of changes, including the upcoming switch to HPV-based primary screening.

STC 02 I-06

PRACTICAL EXAMPLES OF CLINICAL AND CERVICAL SCREENING PROGRAM AUDIT

P. Pearmain

Public Health England (United kingdom)

Background / Objectives

The English Cervical Screening Programme is fully state-funded and provided as a core part of the National Health Service. A formal external quality assurance (QA) programme has been in place since around 1998. Since 2013, the programme management and QA function has become the responsibility of Public Health England, an operationally autonomous executive agency of the Department of Health. A national review of the external QA programme has been ongoing since April 2014 following an external independent review⁽¹⁾.

Methods

The presentation will give examples of the comprehensive approach to cervical screening QA in the English Cervical Screening Programme and the new arrangements for QA in place from April 2016.

Results

Examples of the results achieved by the QA of cervical screening programmes will be discussed.

Conclusion

Robust quality assurance mechanisms are an essential requirement of implementing and running high quality cancer screening programmes.

References

⁽¹⁾ Bentley C, External Review of Quality Assurance for NHS Screening Programmes, Public Health England, Jan 2015, <https://www.gov.uk/government/publications/nhs-screening-programmes-external-review-of-quality-assurance-services>

STC 02 I-07

PRACTICAL EXAMPLES OF CLINICAL AND CERVICAL SCREENING PROGRAM AUDIT - ITALY

N. Segnan

Cancer Epidemiology Unit - University Hospital "Città della Salute e della Scienza di Torino", Turin

Background / Objectives

The new algorithm for Primary screening with DNA-HPV test entails cytology triaging. The cytologist is aware that Pap tests are from a selected population at risk of disease and is therefore more exposed to a risk of overdiagnosis rather than false negatives. A negative result of the Pap test does not refer the woman to the normal screening interval, but to control at 1 year with HPV. The task of the Pap test triage is to bring the specificity of screening with HPV testing to acceptable levels, that means to distinguish, among women already selected by an extremely sensitive test, those who have obvious cytologic atypia and thus a greater risk of disease.

Methods

The Italian Group for Cervical Screening (GISCi) determined quality control recommended actions: (1) Assessment of the distribution of cytological diagnosis; (2) Calculation of the PPV for CIN2 + both total (ASC-US) and for every individual cytological diagnostic category; (3) Peer-review of normal and difficult cases; (4) systematic review of negative cytologies that at 1 year control show CIN 2 or more severe lesion (CIN2 +); (5) Fast rescreening of negative tests; (6) Adoption of a reporting system and uniform use of diagnostic criteria; (7) Circulation of standard set of triage Pap tests; (8) Comparative seminars, also with digital images, on complex cases of cytology triage. Moreover, quality indicators were set: (1) Percentage of abnormal cytologies (ASC-US); (2) Positive Predictive Value for CIN2+; (3) Percentage of positive Pap tests; (4) PPV of HPV+/citology+ for CIN2+ lesions; (5) Detection rate of histological lesions CIN2+ at recruitment; (6) Interval between test and invitation to 1 year repetition.

Conclusion

The percentage of positive tests showed high variability in pilot-studies, probably due to inexperience but also to adjustment of TBS 2001; standard will probably be established between 25% and 35%. In screening with HPV as primary testing, PPV for CIN2 + cytology triage must be

significantly higher than the PPV of cytology screening with the Pap test as a primary test, since in the latter the abnormal cytology often come from women HPV HR-negative. The extensive automation of the molecular and the marked decline in cytology should result in a reduction of the intervals between tests and reports.

References

AAVV. [Triage cytology in cervical screening programs using HPV test for primary screening]. Italian. Italian Group for Cervical Screening. 2013. www.GISCI.it

MSS 01 A-01

HPV BASED SCREENING: PREREQUISITES FOR BUILDING AN EFFECTIVE PROGRAM

A. Anttila

Finnish Cancer Registry, Helsinki (Finland)

Background / Objectives

Organization of services is crucial when considering introduction of primary HPV screening.

Methods

The presentation discusses organizational and implementation issues of primary HPV screening as presented and recommended in the European guidelines Supplement (1).

Results

Key elements of organized screening programmes with primary HPV testing include:

- Adopting a well-defined, evidence-based screening policy
- Establishment of an autonomous, accountable team responsible for programme coordination and provision and quality of the screening services
- A call–recall system for inviting all eligible women to attend screening and for recall of women to repeat examinations or to additional examinations to assess abnormalities detected in screening
- Quality-assured detection, diagnosis and treatment services in all steps
- Centralized data systems to run the programmes
- Regular monitoring based on the centralized data systems
- Screening databases linked to population, cancer, mortality, and vaccination databases and to other relevant registers in health services. These results should be used to prepare regular evaluation, and information to population at large and the various stake-holders.

In addition the guidelines Supplement recommends evidence-based strategies to improve the current attendance to the existing cervical cancer screening programmes.

Conclusion

Many European countries do not yet have a population-based screening programme for cervical cancer, but service has been provided historically mainly through opportunistic practices. In several countries underperforming of cytology-based programmes have also been reported, indicating e.g. lack of coherence within the screening and clinical management services or very low level of participation of the targeted population. These conditions cause challenges in organizing the screening services appropriately.

References

Anttila A, Ronco G, Nicula F, Nieminen P & Primic Žakelj M (2015). Organization of cytology-based and HPV-based cervical cancer screening. S2. In: European guidelines for quality assurance in cervical cancer screening. Second edition, Supplements. Anttila A, Arbyn M, De Vuyst H, Dillner J, Dillner L, Franceschi S, Patnick J, Ronco G, Segnan N, Suonio E, Törnberg S & von Karsa L (eds.). Office for Official Publications of the European Union, Luxembourg, pp. 69–108.

MSS 01 A-02

BUILDING A SUSTAINABLE OPERATIONAL MODEL: TARGET POPULATION, AGE TO START AND STOP, INTERVAL, VACCINE STATUS

M. Elfström

Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Regional Cancer Center of Stockholm-Gotland (Sweden)

Background / Objectives

The pooled analyses of the European primary HPV screening trials have demonstrated a protective effect of HPV-based screening against the hardest endpoint, cervical cancer. Recent supplements to the European guidelines have provided updated guidance on HPV-based screening, stressing the importance of implementing in the context of an organized program. Moving towards a sustainable operational model requires an organized, quality-assured approach to implementation. The pooled analyses of the European primary HPV screening trials have demonstrated a protective effect of HPV-based screening against the hardest endpoint, cervical cancer. Recent supplements to the European guidelines have provided updated guidance on HPV-based screening, stressing the importance of implementing in the context of an organized program. Moving towards a sustainable operational model requires an organized, quality-assured approach to implementation.

Methods

Pilot programs for HPV based screening have been on-going in various settings. Examples of such efforts, including the randomized implementation of HPV-based screening in the organized cervical cancer screening program of Stockholm County, will be used to discuss issues related to guidelines and program structure as well as strategies for monitoring and evaluation.

Conclusion

While pilot programs for HPV-based screening have generally demonstrated non-inferior participation as compared to cytology-based screening, roll-out methods and target populations have differed making it somewhat difficult to compare across settings. Continued monitoring and evaluation of implementation efforts will be needed.

MSS 01 A-03

How to best reach women in screening practice?

M.K. Leinonen

Oslo University Hospital, Cancer Registry of Norway, Department of Research (Norway)

Background / Objectives

High coverage is essential for an effective screening programme but not all women will ever be screened. It is important to distinguish between women who have made a permanent commitment not to get screened, from those who hold positive attitudes to screening but for some reason fail to participate. Studies on factors related to non-participation may help to identify barriers in screening, and knowledge together with new technologies may help to overcome these barriers.

Methods

Our experience is based on 1.3 million women aged 26–69 years living in Norway as of December 31, 2012. We used several national registers to obtain individual sociodemographic and health related data. We defined non-participants as no Pap smear registered within the last screening interval. Non-participants were randomized to receive a self-sampling device (n=729) or a second reminder letter (n=2539).

Results

34% of women were non-participants. Known risk factors for HPV infection such as unemployment, low education, low income and being an immigrant were barriers to screening in a country where organized programme and equal access to health care exist. Self-sampling increased screening attendance from 22%, in the control group, to 33% in the self-sampling group.

Conclusion

In general, socioeconomic inequalities with regard to screening participation exist only in countries with opportunistic screening. Thus, an organized nationwide screening, when offered free of charge, seems to reach women at a higher risk of cancer who are less likely to participate spontaneously.

Postal or telephone reminders and pre-assigned appointment times and locations in the invitation letter are means to reach more woman in a screening practice. Furthermore, invitation sent by the

general practitioner (GP) increases screening uptake compared to an invitation letter from a screening organization.

Self-sampling increases screening participation and might be even more effective than a reminder in attracting underserved women. One plausible explanation is that immigrants are overrepresented among non-participants and they face special barriers to screening some of which are religious, cultural and language related.

More than half of screening non-participants visit their GP regularly. This represents a good opportunity for improving screening coverage. It requires, however, that GPs actively encourage woman to screening uptake and offer self-sampling devices to those who don't comply with a pelvic examination.

Women who don't regularly attend screening are characterized by diversity. To reach the maximal screening coverage requires various approaches fitting to the characteristics of both the programme and the target population.

MSS 01 A-04

Making the right choice from options for HPV testing

J. Cuzick

Queen Mary University of London (United Kingdom)

Background / Objectives

Several HPV tests have been shown to have good performance as screening or triage tests. Most studies of HPV testing have been conducted in either ThinPrep Preservcyt medium or STM collection medium. Increasingly SurePath is now being used for liquid based cytology and there is a need to evaluate the performance of HPV testing assays in this collection medium as well.

Methods

The data on different HPV tests in different media will be reviewed. We focus attention on the range of Predictors studies where up to 6 different tests have been compared both in a screening and triage context and especially on Predictors 4 which studied samples taken in both Preservcyt and Surepath in the 630 women attending for colposcopy due to an abnormal cytology. Two samples were taken from each woman and stored in 20ml of ThinPrep and 10 ml SurePath. The order in which the collection media were used was randomised (319 SurePath first and 311 ThinPrep first). HC2 results were only available for the second part of the study (n=344).

Results

		All (N=630)	CIN3+ (N96)	CIN2+ (N176)	CIN2+ (N454)
Test	Assay	% Positive	Sensitivity	Sensitivity	Specificity
HC2 (Qiagen) (N=344)	ThinPrep/SurePath	84%/78%	0.98/0.98	0.97/0.96	0.21/0.28
Onclarity (BD)	ThinPrep/SurePath	77%/78%	1.00/1.00	0.97/0.97	0.31/0.29
RealTime	ThinPrep/SurePath	76%/71%	0.99/0.97	0.95/0.92	0.32/0.37

(Abbott)					
Sirocco (Genera) (N=585)	ThinPrep/SurePath	79%/80%	0.96/0.96	0.93/0.94	0.26/0.25
APTIMA (Gen- Probe) (N=613)	ThinPrep/SurePath	77%/72%	0.99/0.98	0.97/0.92	0.31/0.36
OncoHealth	ThinPrep/SurePath	57%/48%	0.58/0.55	0.60/0.52	0.45/0.54

These results will be compared to other recent comparisons between HPV tests and transport media.

Conclusion

The histologic abnormalities were 96 CIN3+ (15%), 80 CIN2 (13%), 122 CIN1/HPV (19%), 332 (53%) normal or no biopsy. HPV positivity was lower in SurePath except for the BD and Genera tests (Table). There were always more SurePath negative discordant pairs (ie T pos/Sneg / vs T-neg /S-pos/) and quantitative readings were lower.

MSS 01 A-05

STRENGTHS AND WEAKNESSES OF THE LOGISTICAL IMPLEMENTATION: METHODS, PROTOCOLS, ASSESSMENT, BARRIERS, ACCESS AND COMPLEXITY

N. Segnan, F. Di Stefano

Cancer Epidemiology Unit - University Hospital "Città della Salute e della Scienza di Torino", Turin (Italy)

Background / Objectives

Screening with HPV testing can reduce by 60%-70% the incidence of invasive cervical cancer than screening with Pap test¹. The protocol for HPV-DNA-based primary screening for cervical cancer was suggested by a Health Technology Assessment Report² in 2012. The new algorithm requires a structured investment/disinvestment process. In Italy, a research project has been carried out with the main objective of developing a shared methodological approach to address the introduction of routine HPV-DNA testing for primary screening.

Methods

A survey was carried out, through a questionnaire sent to managers of all Italian regional screening programs, in order to collect information about strategies for implementation of HPV-DNA-based primary screening. It was also performed a systematic review of the literature concerning recommendations, guidelines, HTA reports, indicators, criteria of centralization, disinvestment plans. Data were analyzed by a Working Group composed of Italian screening experts. Criticalities were discussed and consensus was found on suggestions for screening managers involved in implementation.

Results

Nine regional programs answered to the questionnaire. Most of respondent screening programs chose to plan a transitional phase of several years to allow the adjustment of the volume of activity in the transition from the three-year screening interval to the five-years one. All respondent Regions planned the centralization of execution of molecular tests, as well as training of professionals and information to women. According to the cost analysis performed by the Working Group, HPV-DNA-based screening allows to save resources if a reduction of personnel is feasible. In 2012, in Italy, 131,155 women were screened with HPV test³. As an example, in the Piedmont Region, in 2014, 25,289 DNA-HPV tests were performed, out of 185,144 tests⁴.

Conclusion

According to the results of this project, it appears to be very important to regulate activity volumes in the transition, by modulating the flow of access to the new test. This entails the co-existence of two screening pathways for a period and a fair and objective selection method for women. Another key point is the centralization of executions of molecular tests, that showed to lead also to cytology centralization. Centralization and efficiency strategies must include human resources reorganization, that may represent a tricky issue but showed to be less difficult in contexts with shortcoming of personnel.

References

1. Ronco G, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet* 2014; 383: 524-32
2. Ronco G, et al. Health Technology Assessment Report: HPV-DNA-based primary screening for cervical cancer precursors. *Epidemiol Prev* 2012; 36 (3-4) suppl 1: e1-72
3. Zappa M, et al. The national Centre for Screening Monitoring. Eleventh Report. *Epidemiol Prev* 2015; 39(3) Suppl 1: 1-125
4. AAVV. ["Prevenzione Serena". Results, research, quality. Results 2013 – Preliminary data 2014]. Centre for Cancer Epidemiology and Prevention in the Piedmont Region. Turin, 2015. Italian

MSS 01 B-02

IMPLEMENTATION OF PRIMARY HRHPV TESTING FOR CERVICAL CANCER SCREENING IN THE NETHERLANDS

N. Van Der Veen¹, E. Brouwer¹, W. Rodenburg², M. Carpay¹, N. De Jongh¹, B. Hoebee¹

¹National Institute for Public Health and the Environment, Centre for Population Screening, Bilthoven, the Netherlands (Netherlands), ²National Institute for Public Health and the Environment, Centre for Health Protection, Bilthoven, the Netherlands (Netherlands)

Background / Objectives

The cervical cancer screening in the Netherlands will switch from cytology-based screening to primary hrHPV screening with cytology triage in 2017. The change was prompted by Dutch Health Council recommendations in 2011 to improve population screening for cervical cancer. The Health Council advised on hrHPV test criteria, choice of triage, screen intervals and methods to increase uptake using self-sampling for non-responders¹. The route to implementation of these major changes in the Dutch national population screening programme in the Netherlands will be presented.

Methods

A feasibility study on implementation of the hrHPV screening and the self-sampling for non-responders was performed (2010-2012) through a collaborative effort by various stakeholders². The study documented coordination, organisation and cost aspects for the primary process, laboratory QA, monitoring, evaluation and communication to professionals and population. The Dutch government approved implementation based on the results. Currently, we are detailing the practical implementation of quality requirements, communication materials, tendering procedures and IT configuration.

Conclusion

- Attention for the support of stakeholders is essential for successful implementation.
- The option for self-sampling and the 10 year screen interval provided prerequisites on applicable HPV tests.

- International guidelines³ for HPV DNA tests were a valuable resource for test selection.
- International guidelines for self-sampling are lacking and are desired.
- In addition, collaborative, international criteria for monitoring and evaluation, and laboratory QA are needed.

References

¹ Health Council of the Netherlands. Population screening for cervical cancer. The Hague: Health Council of the Netherlands, 2011; publication no. 2011/07.

² Feasibility study for improvements to the population screening for cervical cancer, 2013, van der Veen N, Carpay MEM, van Delden JA, Brouwer E, Grievink L, Hoebee B, Lock AJJ, Salverda-Nijhof JGW. http://www.rivm.nl/Documenten_en_publicaties/Wetenschappelijk/Rapporten/2014/september/Feasibility_study_for_improvements_to_the_population_screening_for_cervical_cancer_2013

³ Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M, Bosch FX, Cuzick J, Dillner J, Heideman DA, Snijders PJ. *Int J Cancer*. 2009;124(3):516-20.

MSS 02-01

TRIAGING METHODS AFTER A POSITIVE HPV TEST FOR CERVICAL CANCER SCREENING: CYTOLOGY

P. Sasieni

Queen Mary University of London (United Kingdom)

Background / Objectives

As is well known, HPV testing is sensitive but not very specific for high-grade Cervical Intraepithelial neoplasia (CIN) whereas high-grade cytology is specific but not very sensitive. By using the more sensitive HPV test as the primary screen and triaging with the more specific test (cytology) one should be able to maintain much of the added sensitivity of HPV testing without referring too many additional women to colposcopy. Ideally triage would divide screen positive women into two groups those who should be referred immediately to colposcopy and those who can be discharged back to routine screening. In practice the only way to gain in sensitivity is to offer early recall to those who test negative on triage. The standard approach is to refer all HPV positive women with any cytological abnormality to colposcopy and to recall those who have normal cytology at 6 or 12 months.

Methods

Whilst it is well known that, in the context of primary screening, cervical cytology is not the same in all laboratories there has been far less acknowledgement of the heterogeneity of cytology when it comes to HPV triage. Here we will look at the sensitivity and positive predictive value of using cytology (with different) cut-offs for HPV triage from studies carried out in different countries.

Although cytology is the norm for HPV triage, there is much interest in using molecular biomarkers either instead of or as an adjuvant to cytology. When used as an adjunct, most researchers have focused on improving the sensitivity of immediate referral for high-grade CIN (or CIN3+). In this talk I will argue that one should consider all those not referred immediately to colposcopy as triage-negative but should be willing to set a variable time for next screen depending on the result of all screening (and triage) tests. Additionally, I will argue that although the threshold for immediate colposcopy should be determined in terms of CIN3, the time to next screen should be set to try to equalise the risk of developing invasive cervical cancer in the interval.

Results

Cytology triage of positive HPV depend on the quality and threshold of the cytology. Systematic reviews should not assume that performance is homogeneous.

It is rational to re-screen cytology normal women sooner if they are HPV16/18 positive than if they only have other high-risk HPV types. Women with high-grade cytology should be referred regardless of HPV type.

Conclusion

Triage protocols may need to be country-specific and should focus on both identifying those benefitting from immediate colposcopy and on extending the screening interval to as long as is safe in those not offered in immediate colposcopy. One size does not fit all.

MSS 02-02

P16 for triage of HPV-positive women

N. Wentzensen

**Division of Cancer Epidemiology and Genetics National Cancer Institute -
Bethesda (United States of America)**

HPV-based cervical cancer screening requires triage markers to decide who should be referred to colposcopy. One candidate triage marker is detection of p16- or p16/Ki-67-stained cells (dual stain) in cytology. p16 and the dual stain have been evaluated for triage of ASC-US and LSIL cytology, for primary screening, and more recently for triage of HPV-positive and HPV-positive/cytology-negative women. A study at Kaiser Permanente Northern California (KPNC) showed that the dual stain assay can be implemented after limited training with high reproducibility. Furthermore, automated evaluation approaches are currently being developed. A prospective evaluation of p16 in an Italian cervical cancer screening trial showed that p16 is a viable option for triage of HPV-positive women, possibly allowing extending follow-up intervals of p16-negative women. Similarly, in an evaluation of the dual stain among women undergoing HPV-cytology co-testing at KPNC, dual stain-positive women had a risk of precancer higher than the colposcopy referral threshold while the risk among dual stain-negatives was below the threshold for a 1-year repeat test. Similar results were observed for HPV-positive/cytology-negative women in the same population. These findings suggest that the dual stain could be an effective triage strategy for HPV-positive and HPV-positive/cytology-negative women.

MSS 02-04

Viral Methylation

A.T. Lorincz

Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London. (United Kingdom)

Background / Objectives

DNA methylation plays a crucial role in activating and silencing genes during normal development. Tumor virus genomes are subject to selective differential methylation with important regulatory consequences. The role of methylation in hrHPV is explored from the perspective of regulation and potential for diagnostic assays.

Methods

Review of published scientific HPV methylation papers in the context of recent research in the laboratory of the author.

Results

HPV16 has ~113 dispersed CpG sites, many of which are differentially methylated. Increased methylation of the L1, L2 and E2 genes is consistently associated with cervical carcinogenesis. Methylation values of some CpG sites in the L1-L2 regions in normal tissues typically range from 0 to 10%, with persistent infections showing higher methylation than transient infections. Methylation values in CIN2/3 are significantly elevated compared to normal, while in cancers methylation levels often reach 50 to 100%. Methylation levels are strongly associated with HPV integration. In contrast to the L1-L2 regions the CpGs in the URR are usually unmethylated in normal and CIN specimens. However, E2 protein binding sites can show increased methylation in cancers, which facilitates continued production of HPV16 E6-E7 oncogenes. Methylation patterns in other hrHPVs resemble HPV16, being significantly higher in CIN2+ compared to <CIN2. Interestingly methylation of L1-L2 in single infections of HPV18, HPV31 or HPV45 in CIN2+ were higher than when present as combinations with HPV16, suggesting that HPV16 is usually the driver virus and is more often targeted by the cellular methylation machinery.

Conclusion

HPV methylation patterns are complex and strongly associated with neoplasia. Similar methylation patterns have been shown in HPV16, HPV18, HPV31, HPV33, HPV45, HPV52 and HPV58 and may be characteristic of most or all hrHPVs. There is a typical progression of methylation level with persistence and carcinogenic change. Development and validation of robust routine methylation assays may allow better disease risk profiling of hrHPV+ women.

MSS 02-06

HPV16/18 genotyping to triage women with ASC-US or LSIL: a systematic review and meta-analysis

M. Arbyn¹, L. Xu¹, F. Verdoodt², N. Wentzensen³, J. Gage⁴, J. Cuzick⁵, J. Belinson⁶, M. Khan⁷

¹Unit of Cancer Epidemiology/Belgian Cancer Centre, Scientific Institute of Public Health, Brussels (Belgium), ²Danish Cancer Society (Denmark), ³Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda (United States of America), ⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda (United states minor outlying islands), ⁵Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London (United kingdom), ⁶Preventive Oncology International Inc. and The Cleveland Clinic, Women's Health Institute, Cleveland, Ohio (United States of America), ⁷Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, Alabama (United States of America)

Background / Objectives

The underlying risk of cervical precancer in women with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous lesions (LSIL) should determine management. Genotyping for the most important carcinogenic HPV types (HPV16 and 18) could identify those women at highest risk requiring colposcopy or more intensive follow-up.

Methods

A meta-analysis was performed to assess the diagnostic accuracy of genotyping for HPV16, HPV16 & HPV18 (HPV1618) and for high-risk types (hrHPV) to detect prevalent cervical intraepithelial neoplasia, grade 2 or 3 or cancer (CIN2+, CIN3+) in women with ASC-US or LSIL cervical cytology. A literature search was performed in three electronic databases to identify eligible studies. Authors were contacted to request additional non-published data. Data pooling was performed using a bivariate normal model designed for diagnostic test accuracy, taking the intrinsic negative correlation between sensitivity and specificity into account. The clinical utility of the triage strategies were illustrated by pretest-posttest probability (PPP) plots.

Results

Twenty-three studies evaluating 15 different HPV assays met criteria for inclusion. The pooled sensitivity of HPV16 genotyping to detect CIN2+ was 54% (95%CI [CI]: 50-58%) and 50% (CI: 47-54%) in ASC-US and LSIL, respectively. The pooled specificity was 86% (CI: 83-89%) and 83% (CI: 80-85%), in ASC-US and LSIL, respectively. The sensitivity was 10-14% higher and the specificity was 3-4% lower for the detection of CIN3+. Adding HPV18 increased sensitivity for CIN2+ compared to testing only for HPV16: ratios of 1.10 (CI: 1.03-1.19) in ASCUS and 1.11 (CI: 1.04-1.19) in LSIL. Adding HPV18 significantly decreased the specificity: ratio of 0.96 (CI: 0.94-0.97) for ASC-US and 0.93 (CI: 0.91-95) for LSIL. The gain in sensitivity and the loss in specificity were similar for detection of CIN3+.

Conclusion

The triage performance of HPV genotyping tests was demonstrated in PPP plots with predefined local decision thresholds. A post-test risk above the referral decision threshold suggests immediate referral to colposcopy while a post-test risk under the threshold suggests increased surveillance or release to routine screening. The average risk of underlying CIN3+ (PPV) was 17% and 19% in HPV16/18-positive women with ASC-US or LSIL, respectively. The risk of CIN3+ among women not carrying HPV16 or 18 was 2% for ASC-US and 4% for LSIL. Being HPV16/18 negative but positive for other hrHPV types is associated with a risk of CIN3+ of 4% and 5% for ASC-US and LSIL, respectively. Whether this risk is sufficiently low to avoid colposcopy and to recommend delayed retesting depends on local guidelines.

MSS 03-01

UPDATE ON THE EVIDENCE AND THE TRANSITION TO HPV SCREENING

G. Ronco

Centre for cancer prevention (CPO) (Italy)

Results

Many double-test studies showed that HPV testing is more sensitive but less specific than cytology for detecting high-grade cervical intraepithelial neoplasia (CIN). Four randomized controlled trials (RCTs) showed reduced detection of high-grade CIN in a second round after HPV-based than after cytology-based screening. This proves that HPV-based screening allows earlier diagnosis of persistent high-grade CIN. A pooled follow-up of these 4 RCTs (176,464 women followed-up for a median 6.5 years) provided direct evidence of greater protection against invasive cervical cancer (ICC): the ICC incidence ratio (HPV vs. cytology arm) was 0.60 (95% 0.40-0.89) considering all the follow-up and 0.45 (0.25-0.81) considering only the period ≥ 2.5 years from recruitment. There was no heterogeneity between studies as for the effect on cancer incidence but there was ($p < 0.0001$) as for the biopsy rate, that was double with HPV than with cytology in the in the only RCT that used direct referral of all HPV+ women to cytology but similar in the remaining 3 that applied a triage. The cumulative incidence of ICC was 8.7×10^{-5} within 5.5 years after a negative HPV test, about half than that within 3.5 years after normal cytology (15.4×10^{-5}). This shows that the screening interval can be safely prolonged to 5 years when it is 3 years with cytology. Longer follow-up of high-grade CIN suggests that the interval could be safely prolonged to 10 years when the interval with cytology is 5. Data from one RCT (but not from another one) are consistent with greater over-diagnosis of spontaneously regressive CIN in younger women. However, the greatest gain in protection with HPV was at age 30-34 years.

Following these results, a pilot study of HPV-based screening routine implementation is almost concluded in the UK. A decision to move to HPV in routine activity has been taken in the Netherlands, in Sweden and in Italy. In Italy 8% of women invited in organised cervical screening programmes in 2012 were invited for HPV, increasing to 10% in 2013 and 13% in 2014.

MSS 03-02

Changes in screening approaches in vaccinated populations (Compass)

**K. Canfell¹, P. Castle², M. Caruana², V. Gebiski³, J. Darlington-Brown²,
C. Jennett², S. Heley⁴, J. Brotherton⁴, D. Gertig⁴, M. Saville⁴**

¹Cancer Research Division, Cancer Council NSW, Sydney, 2011, NSW, Australia (current affiliation) and School of Public Health, University of Sydney, NSW, Australia (Australia), ²Cancer Research Division, Cancer Council NSW, Sydney, 2011, NSW, Australia (current affiliation) (Australia), ³NHMRC Clinical Trials Centre, University of Sydney, 2050, NSW, Australia (Australia), ⁴Victorian Cytology Service Inc., Carlton, 3053, Victoria, Australia (Australia)

Background / Objectives

Compass is a large scale randomised controlled trial of alternate methods of cervical screening in a heavily HPV-vaccinated population in Australia. It is also designed as sentinel experience for the transition of the National Cervical Screening Program in Australia which will transition in 2017 to 5-yearly primary HPV screening.

Methods

In Compass, women aged 25-69 years in the state of Victoria are randomised to 5-yearly HPV vs. 2.5 yearly liquid-based cytology (LBC) screening. Women presenting for screening or follow-up are consented by a primary practitioner and an LBC sample is taken, with randomization occurring once the sample is received by the laboratory. Compass has two phases: Phase I (pilot) involves 5,000 women recruited from Oct 2013 - Nov 2014, and the main trial of 121,000 women commenced recruitment in Jan 2015. Preliminary results will be presented.

Conclusion

Compass is already providing critical information on primary HPV screening in a vaccinated population, including data on test positivity rates, CIN2+ and CIN3+ disease detection rates, and colposcopy referral rates for primary HPV and cytology screening, in cohorts offered vaccination and in older unvaccinated cohorts. This trial will provide critical comparative information on HPV vs. cytology screening in vaccinated women.

MSS 03-03

Evaluation of triage markers in the Costa Rica Vaccine Trial

N. Wentzensen

**Division of Cancer Epidemiology and Genetics National Cancer Institute -
Bethesda (United States of America)**

Current triage strategies for primary HPV screening include cytology and HPV genotyping. Other biomarkers, such as p16/Ki-67 dual stain cytology, have shown promise for triage of HPV-positive women. However, increasing prophylactic HPV vaccination alters HPV type distribution and reduces incidence of cervical cancer precursors in the population, which affects performance of cervical cancer screening and triage tests. Therefore, it is important to evaluate triage markers in vaccinated women. The performance of dual stain cytology was evaluated in 1,500 women ages 18-29 from the Costa Rica Vaccine Trial vaccinated against HPV-16/18 or Hepatitis A (HAV). Cytology slides were stained with the CINtec plus dual stain assay. Assay performance for detection of cervical precancer was evaluated stratified by study arm in women who were negative for vaccine types at baseline and who received all doses of the vaccine (according to protocol, ATP). Dual stain positivity was 11.8% among women who received the HPV 16/18 vaccine and 22.9% among those who received the HAV vaccine. Similar sensitivity of the dual stain was observed between the two arms, but specificity was higher in women who received HPV-16/18 compared to women who received the HAV vaccine. Dual stain positivity was 52% among HPV16-positive controls compared with 23% for controls positive for other carcinogenic types ($p < 0.0001$), accounting for the higher specificity in the HPV vaccinated arm, which included few HPV16-positive controls. In this first evaluation of p16/Ki-67 staining in young vaccinated women, we observed good risk stratification of the dual stain assay for cervical precancer. The performance improved in HPV-vaccinated women, supporting further evaluation of the assay for age-appropriate screening of vaccinated populations.

MSS 03-04

Evidence needed to evaluate screening in vaccinated women

J. Berkhof, N. Veldhuijzen

VUMC (Netherlands)

Background / Objectives

For the coming decades, screening remains an important preventative instrument along with vaccination but the need for screening decreases when vaccine uptake increases and vaccines cover a broad spectrum of HPV types. Evidence on how to screen vaccinated women need to be based on real-life data and transparent cost-effectiveness analyses.

Methods

An overview will be given of data sources that can be used to develop screening strategies for vaccinated women. Data sources include: (1) randomized screening trials performed in vaccinated women, (2) screening trials and cohort studies in which the occurrence of prevalent and incident genotype-specific HPV infections and CIN is estimated, and (3) screening and vaccination registries. The information from different sources can be synthesized by means of a model that informs about the cost-effectiveness of different screening strategies. Specifically, it will be illustrated by means of POBASCAM screening trial data collected over two successive screening rounds (resource type 2) how screening lifetime projections of HPV infection and CIN2+ risks can be obtained under different vaccination strategies (first- and second-generation vaccines, adolescent and adult vaccination).

Conclusion

The main questions for public health decision makers are how to set up a screening program for vaccinated cohorts when vaccine uptake is limited, how to screen cohorts with unvaccinated women and women vaccinated with first- and second-generation vaccines, and which events require screening protocols to be updated.

References

SESSION TITLE:

The future of screening: primary HPV testing in increasingly vaccinated populations

MSS 03-05

Role of modeling to evaluate screening in vaccinated women

S. Kulasingam

University of Minnesota (United States of America)

As HPV vaccine coverage increases and demonstrated reductions in cervical precancer and cancer are confirmed, countries will need to reconsider current approaches to screening. Modeling can be used, in conjunction with epidemiologic data, to suggest optimal screening strategies in the era of HPV vaccines. This talk will provide an overview of current issues in determining how best to change screening and will highlight some of the areas of uncertainty using recent data from the US in combination with a simulation model. The areas of uncertainty include how best to account for herd immunity, if at all and whether women will adhere to screening recommendations once vaccinated.

MSS 03-06

SCREENING OF WOMEN HPV-VACCINATED AS GIRLS

E. Lynge

University of Copenhagen, Dept. of Public Health (Denmark)

Background / Objectives

HPV-vaccination offers good protection against HPV-16/18 related high-grade cervical lesions in women vaccinated before sexual debut. In Denmark, vaccination of girls started with those born in 1993, and 80% received at least one dose. These women are about to enter screening age starting at 23 years in Denmark. The challenge now is to optimise screening for these women.

Methods

We combined screening data for a previous generation of unvaccinated women with data from vaccination trials (1) to estimate the expected screening outcome for vaccinated women (2). We furthermore searched the literature for data on expected outcome of various screening scenarios.

Results

In the unvaccinated generation screened at age 23 years, 8.7% had ASCUS+ and 1.5% had CIN2+. In women vaccinated as girls these percentages were expected to decrease to 6.4% and 1.0%, respectively (2). On this background, we decided to undertake a public health trial where women turning 23 years will be randomised. The control arm will be the present screening programme with cytology testing every third year. The intervention arm will be primary HPV-testing; those testing negative will be reinvited after 6 years; those testing positive will have cytology triage. HPV+/cytology+ women will be followed up according to current guidelines in the Danish cytology-based screening programme. HPV+/cytology- women will be reinvited after 3 years.

Conclusion

Our expectation is that the intervention screening will provide HPV-vaccinated women with at least the same protection against CIN3+ as the present cytology screening; their burden of attending screening will be reduced; and the health care costs will be reduced.

References

(1) Munoz N, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, et al. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. *J Natl Cancer Inst* 2010; 102:325-39.

(2) Hestbeck MS, Lynge E, Kragstrup J, Siersma V, Vazquez-Prada Baillet M, Brodersen J. HPV-vaccination and future cervical screening: The interplay between primary and secondary prophylaxis in primary care. *BMJ Open*. 2015 Aug 14;5(8):e007921. doi: 10.1136/bmjopen-2015-007921.

MSS 04-01

How to monitor and assess HPV safety?

P.L. Lopalco

University of Bari (Italy)

Background / Objectives

HPV vaccines are in use since 2006. HPV vaccine safety has been thoroughly assessed both in large trials and in post-marketing studies. Nevertheless vaccine safety concerns are still a potential barrier to successful implementation of HPV vaccination programmes. So far several vaccine scares have been reported throughout the globe and have been effectively amplified by anti-vaccine activists. Most of the times such events have been carefully investigated and any causal relationship with the vaccine has been ruled out. On the other hand, unexpected events coincidentally occurring after HPV vaccination sometime have not been properly assessed contributing to fuel vaccine hesitancy. HPV vaccine safety data and information have been critically reviewed in order to highlight potential pitfalls and to identify good practices for monitoring and assessing HPV vaccine safety.

Methods

Safety of both HPV vaccine products has been assessed during the pre-licensure phase in large clinical trials. Most frequent adverse events (AE) related to HPV vaccines were injection site symptoms and general symptoms like fatigue, headache, and myalgia, all transient and without sequelae. Serious AE have been rare (<0.1%) and no more frequent than in placebo group. Autoimmune and neurological conditions being triggered by HPV vaccination have been investigated but no consistent evidence for a causal association has been found. Nevertheless several rumours on serious AEs following HPV vaccination are reported since vaccination programmes started, including alleged fatal outcomes. Long-term follow up of clinical trials and observational studies using pharmacovigilance databases have shown no difference in death rates between vaccinees and the general population. More recently two neurological conditions (Complex Regional Pain Syndrome – CRPS; and Postural Orthostatic Tachycardia Syndrome -POTS) have been allegedly linked to HPV vaccination in Japan and Denmark. The Pharmacovigilance Risk Assessment Committee at the European Medicine Agency has concluded that “the review found no evidence that the overall rates of these syndromes in vaccinated girls were different from expected rates in these age groups”, acknowledging that no causal relationship exists between such conditions and HPV vaccination.

Conclusion

HPV safety monitoring represents a paramount element of the vaccination programme. So far alleged serious AEs following vaccination have been a serious threat for the programme when such events have been not properly investigated. Enhanced surveillance and prompt response systems must be put in place in addition to existing routine passive pharmacovigilance systems.

MSS 05-02

Efficacy issues: cervical

E. Joura

Medical University Vienna (Austria)

Background / Objectives

The first generation of prophylactic HPV vaccines has provided an excellent and robust protection against infection and disease with HPV 16 and 18. These two types account for approximately 70% of HPV related invasive cancer and 50% of precancer, hence a medical need remained. A prophylactic effect beyond the HPV types included in the vaccines would be beneficial and marketing activities suggested a major difference between the first generation vaccines.

Methods

Available data of the effect on non vaccine types will be analysed. The effect of mixed infections and co-infections with the vaccine types will be demonstrated. Available data on the duration of cross protection will be reviewed.

Results

After correction for co-infections with vaccine types the prophylactic effect against non- vaccine types appears to be moderate and to wane after some years. The ninevalent HPV vaccine demonstrated no efficacy beyond the vaccine types.

Conclusion

Type specific protection with the HPV vaccine types against disease and infection appears to be superior to cross protective effects. With the ninevalent vaccine the broadest protection with a robust duration can be expected. An analyses of the total efficacy irrespective of the HPV type reflects the incidence of infections with various types in the study population and cannot be generalized for the real world populations.

MSS 05-05

IMMUNOGENICITY AND SAFETY OF THE 9-VALENT COMPARED TO THE 4-VALENT HPV VACCINE: RESULTS OF A DOUBLE-BLIND CONTROLLED STUDY IN 16-26 YEAR OLD MEN

P. Van Damme¹, C. Meijer², D. Kieninger³, A. Schuyleman⁴, S. Thomas⁴, M. Baudin⁴

¹Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Universiteitsplein 1, 2610 Wilrijk (Belgium), ²VU University Medical Centre, De Boelelaan 1117, 1084HV Amsterdam (Netherlands), ³Centre for Clinical Trials, Children's Hospital, Universitätsmedizin, Mainz (Germany), ⁴Sanofi Pasteur MSD, Lyon (France)

Background / Objectives

The 9-valent HPV vaccine (9vHPV vaccine) is comprised of VLPs of the 4 HPV types (Type 6, 11, 16, and 18) contained in the quadrivalent HPV (qHPV) vaccine, and VLPs of 5 additional oncogenic HPV types (Type 31, 33, 45, 52, and 58). In men, qHPV vaccine is indicated for the prevention of high grade anal-intraepithelial lesions, anal cancers and genital warts related to vaccine types. The objective of this study is to extend the efficacy findings observed with qHPV vaccine to 9vHPV vaccine by demonstrating that 9vHPV vaccine elicits non-inferior antibody responses compared to qHPV vaccine for HPV types 6/11/16/18 in men 16 to 26 years of age.

Methods

500 boys/men aged 16-26 years were randomized 1:1 to receive 9vHPV vaccine or qHPV vaccine, at Day 1, Month 2 and Month 6 (3 doses). Antibody responses were evaluated at Month 7 by competitive Luminex Immunoassay. Immunogenicity analyses were performed in the per-protocol population. Safety data were collected from Day 1 to Month 7.

Results

The HPV 6/11/16/18 immune responses elicited by the 9vHPV and the qHPV vaccines were comparable and the non-inferiority of the 9vHPV vaccine compared to qHPV vaccine was demonstrated for all 4 types (lower bound of the two-sided 95% confidence interval around post-dose 3 GMT ratio (9vHPV/qHPV vaccine) greater than 0.5). All participants receiving the 9vHPV

vaccine seroconverted for HPV 31/33/45/52/58. The 9vHPV and qHPV vaccines showed comparable safety profiles.

Conclusion

The 9vHPV vaccine is well tolerated and elicits anti-HPV 6/11/16/18 that are non-inferior to those generated by the qHPV vaccine in 16-26 year-old men. These results support extending the efficacy findings with qHPV vaccine to 9vHPV vaccine in this population.

MSS 05-06

REDUCED-DOSE SCHEDULES FOR PROPHYLACTIC HPV VACCINES

A.R. Kreimer

**Division of Cancer Epidemiology and Genetics, National Cancer Institute,
National Institutes of Health (United States of America)**

Background / Objectives

Cervical cancer and other HPV-associated cancers comprise an important public health burden worldwide, with over 600,000 HPV-associated cancers diagnosed each year. Prophylactic human papillomavirus (HPV) vaccination with three doses of commercially available vaccines, the regimen currently approved by the FDA, is highly efficacious in preventing targeted carcinogenic HPV infections and related cervical cancer precursors. In some regions of the world, two-dose vaccination schedules for adolescents have started to be recommended, based on immunobridging studies demonstrating immunologic non-inferiority of two doses in that age group, compared with three doses of the vaccine in the adult women in the phase III efficacy trials.

Methods

The majority of women who are at the greatest lifetime risk for cervical cancer are not being vaccinated because cost and logistical considerations for administering multi-dose vaccine programs continue to impede progress in reducing this now-preventable cancer.

Results

The NCI-sponsored Costa Rica Vaccine Trial (CVT) and the commercially-sponsored PApilloma TRIal against Cancer In young Adults (PATRICIA Trial), both of which tested the bivalent HPV vaccine, showed similar vaccine efficacy over four years among women who received one, two and three doses of the HPV16/18 vaccine. For the quadrivalent HPV, 36-month preliminary analysis of a large, post-licensure trial in India showed similar protection against HPV16/18 cervical infection whether the women received one dose, two doses, or three doses. However, vaccine recipients in these trials were not randomized to receive these fewer doses, and immunogenicity among one-dose recipients was lower than that observed following two- or three-doses. Thus, the level of evidence in support of single-dose HPV vaccination is insufficient to warrant changes in current recommendations for two- or three-dose schedules. Importantly, stable antibody responses have been observed throughout the seven years of follow-up accrued to date in CVT, suggesting durability of responses.

Conclusion

Reduced-dose schedules for prophylactic HPV vaccines will be discussed, as will the need for additional research, such as a direct evaluation of one-dose efficacy of the HPV vaccines.

MSS 05-07

ALTERNATIVE VACCINATION STRATEGIES

M. Elfström¹, F. Lazzarato², S. Franceschi³, J. Dillner⁴, I. Baussano³

¹Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Regional Cancer Center of Stockholm-Gotland (Sweden), ²Department of Translational Medicine, University of Piemonte Orientale Avogadro, Novara, Italy; Unit of Cancer Epidemiology, Department of Medical Sciences, University of Turin, Italy; International Agency for Research on Cancer, Lyon (France), ³International Agency for Research on Cancer, Lyon (France), ⁴Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Discussions on extending catch-up vaccination efforts and including males in vaccination programs have intensified as vaccine programs targeting females have taken hold and evidence has mounted regarding herd protection effects and HPV-associated disease in men. As vaccination strategies further developed, issues related to overall, in-population effectiveness and resilience of the program need to be explored.

Methods

Comparing alternative vaccination strategies using models based on real-life program data can provide strong predictions for anticipated effectiveness. Two main questions are the impact of extending catch-up vaccination to older females and including males in routine and possibly catch-up vaccination efforts.

Conclusion

Evidence from a validated model calibrated to Swedish data suggests that with extended catch-up of females, decreases in the burden of vaccination-preventable HPV infections can be accelerated as compared to routine school-based vaccination of young girls. Including males in routine vaccination increases the resilience of vaccination programs, minimizing the potential loss of effectiveness due to unexpected drops in vaccine coverage.

MSS 05-08

2nd generation of vaccines, implications for screening

K. Canfell

Cancer Research Division, Cancer Council NSW, Sydney, 2011, NSW, Australia (current affiliation) and School of Public Health, University of Sydney, NSW, Australia (Australia)

Background / Objectives

Background: A second generation nonavalent HPV vaccine (HPV9; Gardasil-9) has been recommended for use in the US, approved for use in the European Union and is currently being evaluated in several other developed countries. In females fully vaccinated prior to HPV exposure, HPV9 is expected to protect against ~90% of cervical cancers. HPV9 is being introduced in the context of established cervical screening approaches but the long term implications for screening are unclear.

Objective: This talk will review the issues to be considered with respect to the role of cervical screening in the context of HPV9.

Methods

A number of key considerations, which will differ between countries, will be reviewed including: (1) the timing for expected impact on screening, which in most countries is expected to be a decade or more and will depend on the age at HPV9 vaccination in relation to the starting age of screening; (2) whether tailored screening approaches depending on a woman's vaccination status will be attempted; such 'individualised' approaches will require comprehensive and reliable vaccine registry systems; (3) the impact of vaccine coverage rates and herd immunity effects on decision-making for screening; (4) the population attributable fraction of HPV9-included types to invasive cervical cancer in different regions; and (5) the costs and cost-effectiveness of continuing to deliver comprehensive screening.

Conclusion

Cervical screening will require re-evaluation to determine what screening, if any, will be required in cohorts offered HPV9. It is likely that 'abbreviated' strategies, perhaps involving only one or a few primary HPV screens in a lifetime, will be appropriate in women known to have been vaccinated with HPV9, or in cohorts with high coverage for HPV9 vaccination.

MSS 06 A-01

HPV VACCINATION IN THE UNITED STATES – THE FIRST DECADE

L. Markowitz, H. Chesson, S. Stokley

Centers for Disease Control and Prevention, USA (United States of America)

Background / Objectives

The first human papillomavirus (HPV) vaccine was licensed for use in the United States in 2006. Since introduction of the vaccination program, there have been changes in recommendations, challenges with implementation and evidence of vaccine impact. To review the first decade of vaccination, we summarize policy, program and impact of HPV vaccination in the United States.

Methods

We reviewed policy statements of the Advisory Committee on Immunization Practices (ACIP), information from the National Immunization Survey –Teen, and other published data.

Results

In the United States, routine HPV vaccination has been recommended for girls since 2006 and for boys since 2011. All vaccines are licensed as 3-dose series. ACIP recommends routine vaccination of girls and boys at age 11 or 12 years, and through age 26 for women and age 21 for men if not vaccinated previously. The United States was the first country to include boys/men in their routine program, a decision based in part on cost effectiveness modeling which showed including boys/men was cost effective given the low coverage among girls at that time. While 3 vaccines are licensed in the United States: quadrivalent HPV vaccine (licensed in 2006), bivalent HPV vaccine (licensed in 2009) and 9-valent HPV vaccine (licensed in 2014), through 2014 almost all vaccine used was quadrivalent HPV vaccine. Vaccine uptake has been slower than expected with >1 dose and 3 dose coverage reaching 60.0% and 39.7%, respectively, among 13–17 year-old girls and 41.7% and 21.6%, respectively, among 13–17 year-old boys in 2014. Multiple professional and public health groups are working together to increase provider knowledge and strength of recommendation, which are critical

for delivering vaccine through primary care providers. The introduction of 9-valent HPV vaccine in 2015 raised several questions, including whether to provide 9-valent vaccine to those who had completed the series with quadrivalent or bivalent HPV vaccine. Despite the good safety profile documented in pre- and postlicensure studies, safety concerns continue to be raised both by anti-vaccine groups and the general public. Early impact of HPV vaccination has been demonstrated by decreases in vaccine type HPV prevalence, genital warts and cervical precancer lesions. Modeling studies predict more substantial decreases in these health outcomes in the future, as well as notable reductions in cervical and other HPV-associated cancers.

Conclusion

Despite challenges in implementation of the HPV vaccination program, progress is being made and vaccine impact on early outcomes demonstrated. Multiple partners are working together to raise vaccination coverage in the United States.

MSS 06 A-02

UNDERSTANDING THE SPECIFICITY OF HPV VACCINE INDUCED CROSS-NEUTRALIZING ANTIBODIES

S. Beddows

Public Health England, U.K. (United kingdom)

Background / Objectives

First generation HPV vaccines (Cervarix® and Gardasil®) have demonstrated almost 100% efficacy against the development of cervical cancer precursors associated with the vaccine-incorporated genotypes HPV16/18. One unanticipated benefit of HPV vaccination is that the vaccines also display a degree of cross-protection against closely related genotypes HPV31/33/45. Impact estimates within the context of national HPV immunization programmes corroborate the vaccine and non-vaccine genotype efficacy data derived from clinical trials. Protection against vaccine-incorporated genotypes is likely mediated by neutralizing antibodies, while the basis for cross-protection against non-vaccine genotypes is less certain.

Methods

Review available data on the specificity of the vaccine-induced cross-neutralizing antibody response in relation to cross-protective efficacy against non-vaccine genotypes.

Results

The generation of cross-neutralizing antibodies is a common feature of HPV vaccination with a tendency for Cervarix® vaccinees to exhibit a broader response with greater magnitude than Gardasil® vaccinees. Although such antibodies constitute only a minor component of the antibody reservoir, they are nevertheless detectable in genital secretions of vaccinated individuals. Serum cross-neutralizing antibodies are detectable several years post-vaccination but display age- and dose-dependencies. Emerging evidence suggests that some naturally occurring HPV variants may be differentially sensitive to vaccine-induced cross-neutralizing antibodies.

Conclusion

These observations do not in themselves establish cross-neutralizing antibodies as correlates of cross-protection but suggest such antibodies may be used as surrogates. The broader vaccine-type

protection afforded by second generation HPV vaccines will likely overshadow any benefit due cross-protection, but there will remain large cohorts of vaccinated women worldwide for which cross-protection will continue to play a significant role.

MSS 06 A-03

Long-term protection and real world effectiveness of Gardasil against the most stringent cervical neoplasia end-points

S.K. Kjaer

Dansih Cancer Society Research Center / Dept of Gynecology, Rigshospitalet, University of Copenhagen, Denmark (Denmark)

Background / Objectives

Monitoring of the quadrivalent HPV vaccine (Gardasil) is conducted in the Nordic long-term follow-up (LTFU) study, which is an ongoing extension of the randomized, placebo-controlled, double-blind phase 3 trial (FUTURE 2). The aim was to investigate the effectiveness of the 4-valent HPV vaccine on the incidence of HPV 16/18-related cervical intraepithelial neoplasia (CIN) 2 or worse in 16-to 23-year old women.

Even though the quadrivalent HPV vaccine has shown excellent efficacy in randomized clinical studies, data on real world effectiveness are important. The study aim was to use individual information on HPV vaccination status to assess subsequent risk of cervical lesions among women from the general population in Denmark.

Methods

In a Danish nationwide study of real world effectiveness of Gardasil, we identified all girls and women born in Denmark in 1989–1999 and obtained information on individual HPV vaccination status in 2006–2012 from nationwide registries. Incident cases of cervical lesions were identified by linkage to the nationwide Pathology Data Bank. Incident cases of cervical lesions were identified by linkage to the nationwide Pathology DataBank. We compared vaccinated and unvaccinated girls and women stratified by birth cohort

In the LTFU study, all women in the Nordic part of the FUTURE 2 trial were followed through different national registries (Denmark, Iceland, Norway and Sweden) for effectiveness data. Effectiveness analyses started approximately 2 years following completion of Protocol 015 and has been occurring approximately every 2 years thereafter. The cohort included a total of approximately 2,700 subjects who received qHPV vaccine at the start of Protocol 015.

Results

Risk of CIN2/3 was statistically significantly reduced among Danish vaccinated women in birth cohorts 1991 to 1994 (HR=0.56, (95% CI=0.37-0.84) in the 1991-1992 cohort). The risk of CIN3 was significantly reduced among vaccinated women in birth cohorts 1993-1994 (HR=0.20, (95% CI-0.06-0.71)).

In the analysis of the long-term effectiveness including a follow-up period up to 10 years, there were 1,281 subjects that contributed to the follow-up period out of a total of 1,984 eligible subjects in the per-protocol population. No new cases of HPV 16/18-related CIN 2 or worse were observed.

Conclusion

Already after a limited number of years (~ 6 years) following licensure of the quadrivalent HPV vaccine in Denmark, a reduced risk of cervical lesions is observed at population level.

The quadrivalent HPV vaccine shows continued protection in women through 8 years, with a trend towards 10 years.

MSS 06 A-04

Long-term efficacy of the quadrivalent and bivalent vaccines against CIN3+

J. Paavonen

Helsinki University Hospital (Finland)

Background / Objectives

HPV disease burden is enormous.

Cervical cancer is the major killer of women worldwide.

Oncogenic HPV is the necessary cause of cervical cancer.

The goal of primary prevention of cervical neoplasia is eradication of the disease.

Active follow-up during the global phase III HPV vaccination efficacy trials has shown strikingly high efficacy against the best surrogate disease endpoint, CIN3.

Passive long-term follow-up based on population based cancer registries is needed to answer the question whether the high HPV vaccine efficacy translates into high efficacy against cervical cancer in real life.

Methods

The FUTURE trial (quadrivalent vaccine) population from Finland and the PATRICIA trial (bivalent vaccine) population from Finland were linked to the Cancer Registry of Finland. The trial populations included women enrolled in the vaccine arms, and matched unvaccinated control women not subject to any health care intervention.

The end-point was CIN3+.

The mean follow-up was 5 years after the active follow-up had ended..

Results

The pilot study based on the FUTURE trial demonstrated an incidence of zero in the vaccine arm, and an incidence of 115-116 in the placebo arm or in the unvaccinated control arm.

The corresponding incidence rates among the PATRICIA trial cohorts were 18 in the vaccinated arm, compared to 115 in the unvaccinated control women.

Thus, the vaccine efficacy was 84%.

Conclusion

Active follow-up of phase III trial populations has shown high vaccine efficacy against CIN3, the most stringent surrogate endpoint for cervical cancer.

Cancer registries and other health registries have an important role in monitoring the overall population impact of the HPV vaccines.

The first results from the long-term follow-up demonstrate high vaccine efficacy against CIN3+.

CIN3+ endpoint cases accumulate rapidly, and repeat registry linkage efforts will continue.

SSim 01-01

Natural Acquired Immunity against Subsequent Genital HPV Infection: a Systematic Review and Meta-analysis

D. Beachler, G. Jenkins, M. Safaeian, A. Kreimer, N. Wentzensen

National Cancer Institute (United States of America)

Background / Objectives

Studies have been mixed on whether naturally acquired HPV antibodies may protect against subsequent HPV infection. We performed a systematic review and meta-analysis to assess whether naturally acquired HPV antibodies protect against subsequent genital HPV infection (i.e. natural immunity). Studies have been mixed on whether naturally acquired HPV antibodies may protect against subsequent HPV infection. We performed a systematic review and meta-analysis to assess whether naturally acquired HPV antibodies protect against subsequent genital HPV infection (i.e. natural immunity).

Methods

We searched the Medline and Embase databases for studies examining natural HPV immunity against subsequent genital type-specific HPV infection in females and males. We utilized random-effects models to derive pooled relative risk estimates for each HPV type.

Results

We identified 14 eligible studies that included over 24,000 individuals from 18 countries that examined HPV natural immunity. We observed significant protection against subsequent infection with HPV16 (pooled RR=0.65, 95%CI=0.50-0.80) and HPV18 (pooled RR=0.70, 95%CI=0.43-0.98) in females but not in males (HPV16: pooled RR=1.22, 95%CI=0.67-1.77, p-heterogeneity=0.05; HPV18: pooled RR=1.50, 95%CI=0.46-2.55; p-heterogeneity=0.15). We also observed type-specific protection against subsequent infection for a combined measure of HPV6/11/31/33/35/45/52/58 in females (pooled RR=0.75, 95%CI=0.57-0.92). Natural immunity was also evident in females when restricting to studies that employed neutralizing assays, to those that used HPV persistence as an outcome, and to those that reported adjusted analyses (each p-value<0.05).

Conclusion

HPV antibodies acquired through natural infection provide modest protection against subsequent cervical HPV infection in females.

SSim 01-02

High risk human papillomavirus targets crossroads in immune signalling.

S.H. Van Der Burg

Leiden University Medical Center (Netherlands)

Background / Objectives

High-risk HPVs infect keratinocytes (KCs) and successfully suppress host immunity for up to two years despite the fact that KCs are well equipped to detect and initiate immune responses to invading pathogens. Viral persistence is achieved by active interference with KCs innate and adaptive immune mechanisms. We studied how hrHPV exploits cellular proteins to interfere with innate and adaptive immune pathway signaling.

Methods

An unique model for hrHPV infection, resembling the natural infection with hrHPV as closely as possible, was used for several types of analyses including micro-array, immunohistochemistry, antibody blocking, small inhibitors, siRNA and several biochemical methods to study the expression of immune receptors by KCs, to study the response of infected and non-infected KCs to innate and adaptive stimuli and to unravel how hrHPV interferes with these pathways.

Results

We assessed signaling via the pattern recognition receptors (PRR), interferon gamma and tumor necrosis factor alpha in non, newly, and persistently hrHPV-infected keratinocytes. We found that active infection with hrHPV hampered the relay of signals downstream of these innate and adaptive immune stimuli, thereby affecting the production of type-I interferon and pro-inflammatory cytokines. In depth studies revealed that hrHPV exploits the cellular proteins UCHL1 and IFRD1 to interfere with NFκB signaling. UCHL1 hampered the interferon pathway by interacting with and deubiquitinating K63-linked polyubiquitin chains from TRAF3, resulting in reduced TBK1 – TRAF3 interaction, IRF3 phosphorylation and IFNβ expression. PRR-induced NFκB signaling was also attenuated through binding of UCHL1 to TRAF6, thereby influencing the Ub status of TRAF6. Furthermore, UCHL1 exacerbated NEMO degradation (1). In addition, hrHPV upregulates epidermal growth factor receptor (EGFR) gene and surface expression via the E5, E6 and E7 proteins. EGFR signaling through mTOR, RAF and/or MEK1, resulted in the increased expression of IFRD1, which mediates RelA K310 deacetylation by HDAC1/3 and as a result in the attenuated transcriptional

activity of NF κ B1. As a result, hrHPV also hampered subsequent immune cell attraction and the response of KCs to incoming signals from the immune system (2).

Conclusion

Thus hrHPV exploits the cellular proteins UCHL1 and IRFD1 to evade host innate immunity by suppressing immune signaling induced keratinocyte-mediated production of interferons, cytokines and chemokines, which normally results in the attraction and activation of an adaptive immune response, enabling the virus to persist. It is highly likely that these mechanism also play a role in other viral infections too and even extends to tumors.

References

1. R. Karim, B. Tummers, C. Meyers, C. Backendorf, R. Offringa, G.J.B. van Ommen, C.J.M. Melief, D. Guardavaccaro, J.M. Boer, S.H. van der Burg (2013) Human papillomavirus (HPV) upregulates the cellular deubiquitinase UCHL1 to suppress the keratinocyte's innate immune response. PLoS Pathogens 9(5):e1003384
2. B. Tummers, R. Goedemans, L.P.L. Pelascini, E.S. Jordanova, E.M.G. van Esch, C. Meyers, C.J.M. Melief, J.M. Boer, S.H. van der Burg (2015) The interferon-related developmental regulator 1 is used by Human papillomavirus to suppress NF-kappaB activation. Nature Communications, 6:6537

SSim 01-03

Escape from innate immunity: HPV E6-mediated dysregulation of interleukin-1 β in human keratinocytes

M. Niebler, B. Rincon-Orozco, F. Rösl

German Cancer Research Center, Heidelberg (Germany)

Background / Objectives

An innate immune response is crucial to inhibit pathogen spread until adaptive immunity is activated. Human papillomaviruses are able to inhibit various pathways including interferon signalling, chemokine expression and other cytokines. Interleukin-1 β , for instance, is a pro-inflammatory cytokine which is produced by cells of the monocyte lineage as well as keratinocytes upon infection. IL-1 β is able to activate immune cells and is involved in the up-regulation of adhesion molecules on endothelial cells. Because of its strong pyrogenic effects the production of IL-1 β is tightly controlled on the transcriptional as well as post-translational level. Here, we investigated the influence of the oncogenes E6 and E7 of HPV16 on the expression, post-translational processing and secretion of IL-1 β .

Methods

In our studies we used primary keratinocytes (pK), non-tumorigenic keratinocytes that were retrovirally immortalized by the HPV16 oncogenes E6 or/and E7 (E6, E7 and E6/7 cells) as well as the HPV16-positive cervical carcinoma (CxCa) cell lines CaSki and SiHa for molecular analysis.

Results

Upon adenoviral infection, pK and E7-immortalised cells secrete high amounts of IL-1 β while E6 and E6/7 cells or CxCa cells do not respond. qPCR revealed that in CxCa cells the transcription of the IL-1 β gene is reduced when compared to pK. Conversely, despite similar mRNA levels in immortalised cells, only pK and E7 cells show a constitutive expression of pro-IL-1 β while the protein is degraded in a proteasome-dependent manner in E6-positive cells which is mediated via the ubiquitin ligase E6-AP and p53. Conversely, in E6- and E6/E7-immortalized cells pro-IL-1 β levels were restored by siRNA knock-down of E6-AP and simultaneous recovery of functional p53. Transfection of an E6 expression plasmid into E7 cells lead to a decrease of pro-IL1 β . Treatment of E6 and E6/7 cells with the proteasome inhibitor MG132 was able to restore intracellular pro-IL1 β levels. Mutagenesis analysis of the N-terminal part of pro-IL-1 β led to the identification of several lysine residues responsible for degradation. Currently, a siRNA library screen is conducted to identify factors involved in this process.

Conclusion

In HPV positive cells, IL-1 β is abrogated in two ways: by an E6-mediated post-translational and proteasome-dependent mechanism in immortalised cells and by additional transcriptional silencing in tumor cells. We conclude that the post-translational degradation of IL-1 β upon infection with HPV16 represents an early mechanism of viral immune escape, while the transcriptional silencing of IL-1 β takes place during an additional selection process towards tumour formation.

SSim 01-04

HPV16 E7 PROTEIN CAUSES DYSFUNCTION OF MHC CLASS I TO INDUCE CARCINOGENIC TRANSFORMATION OF VIRUS-INFECTED KERATINOCYTES IN VITRO AND IN VIVO

L. Zhang ¹, F. Zhou ¹, J. Chen ², K.N. Zhao ³

¹Institute of Molecular Virology and Immunology, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, PR China (China), ²School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia (Australia), ³Institute of Molecular Virology and Immunology, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, PR China, 3. Centre for Kidney Disease Research-Venomics Research, The University of Queensland School of Medicine, Translational Research Institute, 37 Kent Street, Woolloongabba, Brisbane, QLD (Australia)

Background / Objectives

Human cells infected with HPVs are able to escape from host immunosuppression leading to the development of cancers. HPV oncogene E7 has been reported to have specific biological features to affect host immune systems such as MHC class I antigen processing and presentation. Our aims are to investigate whether and how HPV16 E7 down-regulates MHC class I to induce carcinogenesis in virus-infected keratinocytes.

Methods

Four established systems: (HPV16 E7-transgenic mouse keratinocytes (E7-KCs), human primary keratinocyte cultures, two-cell co-cultures and mice model were used to achieve our aims.

Results

HPV16 E7 protein inhibits interferon- γ (IFN- γ)-mediated enhancement of keratinocyte antigen processing and T-cell lysis, leading to the blockade of interferon regulatory factor-1 (IRF-1) and TAP-1 expression in E7-KCs. HPV16 E7 prevents IFN- γ -induced phosphorylation of STAT1(Tyr701) to decrease MHC class I antigen presentation through Cytotoxic T lymphocytes (CTLs), leading to escape of IFN- γ -treated E7-KCs from immune surveillance (CTLs-mediated killing) through the

JAK/STAT1/IRF-1 signaling pathway. In human primary keratinocyte cultures, expression of HPV16 E7 significantly increased the activities of PI3K/Akt and MAPK as indicated by increased protein levels of PI3K, p-PI3K, p-AKT and p-MAPK accompanied by a dramatically decreased level of pRb protein. The expression of HPV 16 E7 substantially inhibited expression of IRF-1, STAT1 and PKC δ , in contrast to the marked upregulation of STAT3 and PKC α . HPV16 E7 expression also markedly increased the frequencies of G0/1 cells, leading to an increase in keratinocyte survival/proliferation. Two-cell co-cultures revealed that HPV16 E7-transduced lymphoma cells (E7-EL4) were shown to fuse with mouse primary keratinocytes, leading to distinct changes of the KC morphology within seven weeks. The fusion of E7-EL4 cells with keratinocytes that induced formation of spheroid-like colonies and multiple-cell tumor colonies was associated with upregulated expression of several oncogenes including c-Jun, c-Fos and k-Ras and abnormal expression of several MHC class I molecules. Furthermore, mice subcutaneously challenged with E7-EL4 cells could also develop skin tumors. These skin tumors showed substantial disruption of spectrin/adducin-based cytoskeletons and significantly increased protein levels of p-PI3K/p-AKT.

Conclusion

All data reveal that E7 down-regulated MHC class I to mediate immune escape, which could play a crucial role in human cancers caused by infections with HPVs. E7 oncogene is a potential target for developing immunotherapy of HPV-induced cancers.

SSim 01-05

Human papillomavirus (HPV) downregulates the expression of RIP3 and IFITM1 to resist cell death and cell senescence induced by IFN γ and TNF α

W. Ma, B. Tummers, S. Van Der Burg

Department of Clinical Oncology, Leiden University Medical Center, Leiden, The Netherlands (Netherlands)

Background / Objectives

Basal keratinocytes (KCs) are responsible for renewal of the epithelium and are the target cells for high risk human papilloma viruses (hrHPVs) which may cause KCs to become transformed. The immune system has developed means to counteract infections through several mechanisms, including the suppression of viral spread through the proliferation of infected cells via the production of the effector cytokines IFN γ and TNF α . hrHPV also have the ability to resist cell death and cell senescence induced by IFN γ and TNF α .

Methods

Using an unique system for freshly established or persistent hrHPV infection, we first screened the gene expression related with cell growth and cell death of hrHPV16 positive keratinocytes(HPV+ KC) and normal keratinocytes treated with IFN γ by gene array. Then we checked the target in the TNFR1 and IFN γ R pathway by Western blot and qPCR. SYTOX green assay was applied to check the necroptosis. DNA staining is applied to check the proliferation, as well as qPCR of certain proliferation markers.

Results

We found that hrHPV escapes from IFN γ and TNF α induced cell death by impairing the expression of RIP3 at the gene and protein level. Subsequently, the phosphorylation of MLKL is also reduced. RIP3 is part of the necrosome and as a consequence stimulation of KC and HPV+ KC by BV6 and zVADfmk plus IFN γ and TNF α only marginally induced cell death in HPV+ KC while almost all non-infected KC die. Furthermore, we observed that hrHPV impairs the IFN γ growth inhibitory pathways by targeting the expression of the anti-proliferative gene IFITM1. The expression of IFITM1 was downregulated already at 48 hours after hrHPV infection. In non-infected KCs stimulation with IFN γ and TNF α induced the expression of the antiproliferative gene RARRES and suppressed the expression of the with proliferation associated gene PCNA. However, this was not the case in HPV+ KCs. In order to

recapitulate the effect observed in HPV+KCs, we knocked down IFITM1 in non-infected KCs and showed that this provided resistance to the anti-proliferatory effects of IFN γ and TNF α .

Conclusion

Our study revealed that HPV downregulated the expression of RIP3 and IFITM1 to resist IFN γ and TNF α induced cell death and senescence.

SSim 01-06

HLA CLASS II ANTIGEN EXPRESSION IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CANCER

M. Sauer¹, **M. Reuschenbach**¹, **N. Wentzensen**², **F. Soldano**³, **B. Lahrman**⁴, **N. Grabe**⁵, **D. Schmidt**⁶, **M. Von Knebel Doeberitz**¹, **M. Kloor**¹

¹Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit, German Cancer Research Center (DKFZ), Heidelberg (Germany), ²Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland (United States of America), ³Massachusetts General Hospital, Harvard Medical School, Department of Surgery, Boston (United States of America), ⁴Hamamatsu Tissue Imaging and Analysis Center (TIGA), BIOQUANT, University of Heidelberg, Heidelberg (Germany), ⁵Hamamatsu Tissue Imaging and Analysis Center (TIGA), BIOQUANT, University of Heidelberg, and National Center of Tumor Diseases, Medical Oncology, University Hospital Heidelberg, University of Heidelberg, Heidelberg (Germany), ⁶Institute of Pathology, Viersen (Germany)

Background / Objectives

HLA class I antigen expression on tumor cells is essential for the recognition of tumor antigens by the immune system. HLA class II antigens normally are expressed by professional antigen-presenting cells, but are also reported to be expressed by several tumors of non-lymphoid origin. Strong HLA class II antigen expression has been described for a subset of HPV-associated cervical cancers. To characterize HLA class II antigen expression during HPV-induced cervical tumor development, we examined HLA class II antigen expression in CIN lesions and cervical cancers and correlated HLA class II expression with immune cell infiltration in the lesions and the adjacent stroma.

Methods

FFPE tissue sections of CIN1, CIN2, CIN3 and invasive SCC patients (n=103 in total) were analyzed by immunohistochemical staining with monoclonal antibodies specific for HLA class II antigens (LGII-612.14) and for different T cell markers (CD3, CD8, Foxp3, Granzyme B, CD3 zeta-chain).

Results

HLA class II antigen expression was absent in all samples of normal, non-neoplastic squamous cervical epithelium adjacent to lesions (n=29). However, a strong and uniform staining pattern was found in the columnar epithelium and cells of the squamocolumnar junction zone. HLA class II antigen expression was low in CIN1 (40.9%) and peaked in CIN2 (90.0%), then decreasing again towards CIN3 lesions (71.4%) and cancer (63.6%). In CIN3 and cancers high CD3+ and CD8+ lymphocyte infiltration correlated with lack of or heterogeneous HLA class II antigen expression.

Conclusion

Our results suggest that HLA class II antigens are commonly expressed in precancerous stages and cervical cancers. The low percentage of HLA class II positivity in CIN1 is compatible with the hypothesis that only a subset of CIN1, potentially those originating from the squamocolumnar junction zone, may overexpress HLA class II antigens and tend to progress into higher grade CIN. In later disease stages, HLA class II antigen-positive cell clones may be eliminated in an environment of dense T cell infiltration, which would be compatible with the immunoediting concept.

SSim 02-03

Efficacy of VLP vaccination against papillomavirus-induced skin tumors in the animal model *Mastomys coucha*

S. Vinzon, M. Müller, F. Rösl

German Cancer Research Center, Heidelberg (Germany)

Background / Objectives

The rodent *Mastomys coucha* is naturally and persistently infected with *Mastomys natalensis* papilloma virus (MnPV). The animals are unique in spontaneously developing MnPV-induced benign skin lesions (papillomas, keratoacanthomas) that also can progress to non-melanoma skin cancer. Infection occurs early in lifetime, similarly to cutaneous HPV, and high viral loads can be detected in the skin in older animals, which trigger the onset of papilloma development. Therefore, *M. coucha* represents an excellent model to investigate prophylactic approaches against papillomavirus-caused skin tumors under natural conditions. The aim of this preclinical study is to investigate a virus-like particle (VLP)-based vaccine with respect to the prevention of skin tumors under conditions of primary or already established infection, both in immunocompetent and immunosuppressed animals.

Methods

A papillomavirus-free *Mastomys* colony was raised by hysterectomy of animals from the naturally infected colony. MnPV infected and non-infected *Mastomys* were immunized with MnPV VLPs and subsequently, depending on the group, experimentally infected and/or fed chronically an immunosuppressive diet. Elicitation of protective antibodies was evaluated by VLP-ELISA and a pseudovirion-based neutralization assay.

Results

We found strong and steadily increased L1 antibody levels in immunized animals, while in naturally infected animals immune response against capsids is delayed and only reaches immunization-like levels in older animals. Animals fed with a Cyclosporin A diet did not show a different behaviour in their humoral immune response against either the virus or the vaccine. Our results show that the VLP-based vaccine effectively prevents the appearance of both skin lesions and tumors, irrespective of the infection status of the animal at the time of vaccination. Furthermore, vaccination results in an overall lower viral load in skin when compared to control animals. New data about this model will be presented.

Conclusion

Our findings provide evidence that VLP vaccination elicits an effective immune response even under immunosuppressive conditions and will support the clinical development of potent vaccination strategies against cutaneous HPV infections.

SSim 02-04

ANALYSIS OF PHASE II TRIAL OF A HPV THERAPEUTIC DNA VACCINE, GX-188E, IN PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

3

J.S. Park¹, T.J. Kim², J.K. Lee³, C.H. Cho⁴, S.Y. Hur¹, S.R. Hong⁵, Y.B. Seo⁶, K.S. Park⁷, H.T. Jin⁶, C.M. Kang⁷, Y.Y. Hwang⁷, J.W. Woo⁷, Y.C. Sung⁸

¹Department of Obstetrics and Gynecology, Seoul St Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul (Korea, republic of),

²Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul (Korea, republic of), ³Department of Obstetrics and Gynecology, Korea University Medical Center, Seoul (Korea, republic of), ⁴Department of Obstetrics and Gynecology, Keimyung University School of Medicine, Daegu (Korea, republic of), ⁵Department of Pathology, Cheil General Hospital & Women's Healthcare Center, Dankook University College of Medicine, Seoul (Korea, republic of), ⁶Research Institute, BioDion Inc., Korea Bio Park, Seongnam, Gyeonggi-do (Korea, republic of), ⁷Research Institute, Genexine Inc., Korea Bio Park, Seongnam, Gyeonggi-do (Korea, republic of), ⁸Research Institute, Genexine Inc., Korea Bio Park, Seongnam, Gyeonggi-do/Division of Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang (Korea, republic of)

Background / Objectives

GX-188E is a novel, dendritic cell targeting, DNA therapeutic vaccine encoding for HPV types 16/18-E6/E7 antigens. A previous phase I trial has been reported(1).

Methods

72 patients were enrolled on an open-label, multicenter Phase 2 trial. Eligible patients had biopsy proven CIN3 and HPV16 and/or 18 infection confirmed by PCR. GX-188E was delivered by electroporation at weeks 0, 4, and 12. The primary endpoint was a response defined as histological regression to CIN1 or less at week 20. Safety and Immunogenicity of the vaccine were also assessed. Additionally, patients could be rolled over into a continuation study for follow-up beyond 20 weeks.

Results

72 pts were enrolled. 7 patients were dropped out or were found to be ineligible (data to be shown). 4 patients have not yet reached their 20 week f/u visit. As of January 2016, 61/72 CIN3 patients reached week 20. Of the 61 patients, 52.5% (32/61) regressed to CIN1 or less on histology at week 20. Patients with small lesion at enrollment (<50% of cervix by colposcopic inspection) were more likely to have histological regression (61.8%, 21/34) as compared to patients with lesions >50% (40.7% 11/27). The 1mg dosing group demonstrated a higher histological regression rate (64.5%, 20/31) compared to the 4 mg dosing group (40.0%, 12/30). Moreover, patients in the 1 mg group with small baseline lesions showed a regression rate of (73.3%, 11/15) and this rate increased at week 36 (88.9%, 8/9) in the follow-up study. The most common adverse events were local injection site reactions. Additional detailed analysis of safety and immunologic response assessment is ongoing.

Conclusion

Therapeutic vaccination with GX-188E appears well-tolerated. GX-188E vaccine shows promising activity and therapeutical potential for treatment of CIN3 and warrants continued investigation.

References

(1) Kim TJ, Jin H-T, Hur S-Y, et al. Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. *Nature Communications*. 2014;5:5317. doi:10.1038/ncomms6317.

SSim 02-05

DEVELOPMENT OF A THERAPEUTIC CANCER VACCINE BASED ON p16^{INK4a}

**K. Urban¹, R. Carretero², E.S. Prigge¹, W. Osen³, G.J. Hämmerling⁴,
M. Reuschenbach¹, M. Von Knebel Doeberitz¹**

¹Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), Heidelberg (Germany), ²DKFZ/BHC Joint Immunotherapy Lab, German Cancer Research Center (DKFZ), Heidelberg (Germany), ³Department of Translational Immunology, German Cancer Research Center (DKFZ), Heidelberg (Germany), ⁴Tumorimmunology Program, German Cancer Research Center (DKFZ), Heidelberg (Germany)

Background / Objectives

For the development of therapeutic cancer vaccines, tumor antigens need to be identified that are either specific or aberrantly expressed in tumor cells compared to normal cells. The cyclin-dependent kinase inhibitor p16^{INK4a} is strongly overexpressed in human papilloma virus (HPV)-induced tumors, whereas it is barely detectable in normal tissue. Therefore, it is an established surrogate marker for high risk HPV infections and considered to be an interesting target for therapeutic vaccination in cancers associated with HPV. p16^{INK4a} expression has also been detected in non-HPV-related tumor entities as colorectal and small cell lung cancer, suggesting p16^{INK4a} as a broad tumor associated antigen that is not only specific for HPV-induced cancers.

In a phase I/IIa trial to monitor toxicity and immunogenicity of a p16^{INK4a} peptide vaccine in patients with advanced HPV-associated, p16^{INK4a}-overexpressing cancers, we could show the induction of a humoral and cellular immune response against p16^{INK4a} without any severe vaccine-related side effects.

Presently we are establishing a p16^{INK4a}-positive tumor mouse model in order to explore the effect of a p16^{INK4a}-based vaccine on tumor growth and its potential to be combined with current immunotherapies.

Methods

Pools of long, synthetic peptides covering the whole p16^{INK4a} sequences were injected into female C57BL/6 mice. Establishing the p16^{INK4a}-positive tumor mouse model, C57BL/6 mice were challenged with p16^{INK4a}-expressing TC-1 cells before and respectively after the peptide immunization to analyse the tumor response of a therapeutic and respectively prophylactic vaccine approach.

Results

The p16^{INK4a}-based vaccination with long, synthetic peptides induced an antibody response against p16^{INK4a} detected by ELISA as well as p16^{INK4a}-specific IFN γ -producing T cells measured by ELISpot. We are currently performing the tumor regression and protection experiments with p16^{INK4a}-positive TC-1 tumor cells in C57BL/6 mice.

Conclusion

The established murine system allows to address the question whether a p16^{INK4a}-based vaccine is able to induce the regression of an established p16^{INK4a}-positive tumor and/or to prevent the further outgrowth of a tumor expressing p16^{INK4a}. The generation an effective tumor response against p16^{INK4a} could lead to a new therapeutic approach for HPV-induced cancers as well as for tumors overexpressing p16^{INK4a} independent of an HPV infection.

SSim 02-06

PRECLINICAL PROOF OF CONCEPT OF GTL002, A MULTIVALENT CANDIDATE FOR THE IMMUNOTHERAPY OF HUMAN PAPILLOMA VIRUS HPV16/18/45/31/33/52-INFECTED WOMEN.

M. Esquerré¹, Y. Misseri¹, E. Totain², G. Zingone¹, N. Cohet¹, P. Bridonneau¹, C. Gonindard¹, M. Best¹, A. Navarro¹, J. Morato¹, H. Keller¹, A. Bousquet-Mélou², S. Boullier², M.C. Bissery¹

¹GenticeL (France), ²Ecole Nationale Vétérinaire de Toulouse (France)

Background / Objectives

In this study, we investigate the *in vitro* and *in vivo* activities of GTL002, a novel Human Papilloma Virus (HPV)-specific immunotherapy aimed to treat infection by 6 high-risk HPVs responsible for 85% of cervical cancer worldwide.

Methods

GTL002 is made of 2 recombinant proteins based on GenticeL's vector called Vaxiclase (a modified Adenylate Cyclase protein from *B. pertussis*) comprising modified E7 proteins from HPV16, 18 and 45 (C216) and from HPV31, 33, 52 (C331). Each C216 & C331 protein was produced in *E. coli* using an improved phase I/II process. GTL002's ability to target the integrin CD11b on antigen-presenting cell was assessed by competitive binding using a CD11b/CD18-expressing cell line. The immunogenicity was evaluated in inbred (C57BL/6), outbred (Swiss) mice and in Beagle dogs. Animals are injected intradermally with GTL002, adjuvanted either with co-formulated poly-ICLC, a TLR3 agonist, or with topical application of imiquimod 5% cream, a TLR7 agonist, at the injection site. E7-specific T cell responses were measured by an *ex vivo* IFN γ ELISpot using overlapping peptides covering each of the 6 E7 proteins for restimulation of splenocytes. The cytotoxic T lymphocytes (CTL) were measured by an *in vivo* killing assay and the therapeutic efficacy was evaluated in a TC-1 tumor model in C57BL/6 mice. The T cell and antibody responses were followed in Beagle dogs weekly for 11 weeks.

Results

C216 and C331 proteins are successfully produced after IPTG induction at 10L scale, and purified by chromatography. The production process is transferable and scalable at industrial scale. GTL002 is found to bind to CD11b with a K_i of 22 nM. GTL002 induces HPV16-, 18-, 33-, and 52 E7-specific T cell responses in C57BL/6 mice as well as HPV16 and HPV18 E7-specific CD8+ CTL in mice that are able to

eradicate HPV16 E7-expressing TC-1 tumors. E7-specific T cell responses against HPV16-, 18-, 45-, 31-, and 52 are induced in Swiss mice. In Beagle dogs, administration of GTL002 on day 0, 21 and 42 is well tolerated whatever the adjuvant used. Local reactions are transient. Repeated administrations of GTL002 induce HPV16/18/45/31/33/52-E7-specific T-cell immune responses without increasing reactogenicity. There was a good immune response with both the co-formulated poly-ICLC and topical imiquimod.

Conclusion

The readily scalable and transferable process for commercial development, the broad immunogenicity against the 6 high-risk HPVs, the in vivo HPV16-E7 target cell efficacy and the good tolerance Beagle dogs, all together warrant GTL002 immunotherapy to move to human clinical development.

SSim 02-07

IMMUNOTHERAPY WITH INO-3112 (HPV16 AND HPV18 PLASMIDS + IL-12 DNA) IN HUMAN PAPILOMAVIRUS (HPV) ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCCA)

C. Aggarwal¹, **R. Cohen**¹, **M. Morrow**², **K. Kraynyak**², **J. Bauml**¹, **G. Weinstein**¹, **J. Boyer**², **J. Yan**², **D. Mangrolia**², **S. Oyola**², **J. Lee**², **S. Duff**², **Z. Yang**², **M. Bagarrazi**²

¹University of Pennsylvania (United States of America), ²Inovio Pharmaceuticals (United States of America)

Background / Objectives

Oropharyngeal HNSCCa is frequently associated with HPV infection. DNA-based immunotherapy with plasmids encoding HPV16 and HPV18 E6/E7 antigens has been shown to generate robust immune responses in women with HPV-driven high-grade cervical dysplasia. We hypothesize that immunotherapy with INO-3112 in patients (pts) with HPV-associated HNSCCa will generate robust immunity contributing to disease control.

Methods

This open-label Phase I/IIa trial included adults with HPV-positive HNSCCa. Cohort 1, pts received INO-3112 pre and post-surgery; Cohort 2, pts received INO-3112 after completion of cisplatin based chemoradiation. INO-3112 was delivered intramuscularly followed by electroporation with the CELLECTRA[®] device, every 3 weeks for 4 doses. Pts are followed for 2 years. Primary and secondary endpoints are safety and immune responses. Exploratory endpoint: clinical response.

Results

As of January 2016, 20 pts have been treated. Cohort 1: n=6, Cohort 2: n=14; 18 males, median age 57 years; cancers at base of tongue=7, tonsil=13; never smoker=8; median follow-up of 195 days. INO-3112 was well tolerated, with no treatment related Grade 3 AE, no \geq Grade 4 AEs. The common ($\geq 10\%$) AEs were injection site pain (n=14), injection site erythema (n=4), injection site swelling (n=3), dizziness (n=3), dysphagia (n=2), injection site hematoma (n=2) and candidiasis (n=2). Two unrelated SAEs: Grade 2 post-surgical procedure hemorrhage and Grade 3 acute non-traumatic kidney injury. Enrollment and correlative analysis are ongoing. Among samples tested to date (n=10), as compared to baseline, all 10 evaluable pts showed elevated antigen specific antibody titers. Nine of 10

evaluable pts exhibited increased HPV-specific cellular responses by IFN-gamma ELISpot. Eight out of 9 evaluable pts had HPV-specific CD8+ T cell responses to INO-3112 by flow cytometric analysis and all 10 pts had positive cellular immune responses in at least one assay.

Conclusion

These interim data demonstrate that INO-3112 can be administered safely in pts with HPV-related HNSCCa. All evaluable pts demonstrated humoral and cellular immune response, including generation of HPV-specific CD8+ T cells.

References

This study (NCT02163057) is co-sponsored by the Abramson Cancer Center at the University of Pennsylvania and Inovio Pharmaceuticals.

SSim 03-01

Burden and Epidemiology of HPV 16 infection and related cancers in men and women

S. De Sanjose

Cataln Institute of Oncology (Spain)

Background / Objectives

HPV 16 is the most relevant oncogenic type in humans. Is the msot prevalent in all cancer sites involving HPV and affects both men and women.

Methods

Extraction from the ICO/IARC HPV information center data and additional literature review on the burden of HPV 16 in men and women with and without associated diseases.

Results

HPV16 is detected in about 2.8% of women with a concomitant negative cervical cytology. There is a clear enrichment of HPV 16 when evaluation is done by abnormal results going from 19% in low garde lesions to 55% in invasive cervical cancer cases. Interestingly there is no major differences in these parameters by develop/developing world resgions. Information on genotype distribution in men with and without lesions is limited. Among HPV positive cases, HPV 16 is involved in over 80% of the cancer sites anal, vagina, vulva, penile and head and neck tumors.

Conclusion

Although a large body of published HPV genotype-specific data is currently available showing the relevance of HPV16 above all other HPV oncogenic types, data gaps remain for genotype-specific infection incidence and several world regions with the highest HPV cancer burden.

SSim 03-02

Assessing HPV 16 infection risk in the general population: the value of HPV16 variants

G. Clifford

International Agency for Research on Cancer, Lyon, France (France)

Background / Objectives

Factors that favour a proportion of HPV16 infections to progress to cancer are still poorly understood, but an increasing number of studies have implicated a role of HPV16 variants in cervical cancer risk.

Methods

An HPV variant is a genome defined by a unique combination of single nucleotide polymorphisms (SNPs). A recent proposal defines major variant lineages by a 1.0% difference between full genomes of the same HPV type, with differences of 0.5 to 0.9% defining sub-lineages. Based on whole HPV genome sequencing, HPV16 variants have been classified into four major lineages: (1) A, that includes sub-lineages A1–3 (previously named European), and A4 (Asian); (2) B (African 1); (3) C (African 2); and (4) D (including Asian–American [AA] and North-American [NA]) (Burk et al., 2013).

Results

The distribution of HPV16 variant lineages around the world is highly geographically and ethnically specific, which limits the extent to which data can be easily combined across countries and interpreted soundly. Nevertheless, some consistent findings in case:control comparisons in multiple geographic settings do appear to exist, namely :

D (North American/Asian-American) variant lineages appear to be associated with HPV16 persistence and CIN3+/cervical cancer development (Berumen et al., 2001; Xi et al., 2007; Schiffman et al., 2010; Cornet et al, 2013).

A4 (Asian) variant lineage shows association with cervical cancer, particularly in East Asian populations where they are common (Cornet et al, 2013; Chang et al 2013; Hang et al, 2016).

It is not yet clear which of the many correlated lineage-defining SNPs are at the cause of these differential risks, but work is currently moving to the finer level of genetic analysis by performing whole HPV16 genome sequencing in an appropriately large number of HPV16-positive cases and controls (see 17/06 session: “new advances in genomics”).

To date, little is known about the distribution of variant lineages in HPV16 infection and cancer at other sites of the anogenital tract or head and neck, but there is no striking evidence or biological plausibility why it should be any different from that in the cervix.

Conclusion

Although our findings suggest that HPV16 variant analysis has no clinical application at present (all variant lineages are commonly found in cervical cancer), understanding the genetic basis of differences in the carcinogenicity of HPV16 variants may help us unravel important biological and/or immunological interactions between virus and host that could lead to better tools to control HPV infection and its malignant consequences.

SSim 03-03

Appropriate methods of detection and clinical utility of HPV 16 identification

F. Carozzi

Institute of cancer prevention (Italy)

Background / Objectives

HPV16 is the most frequent HPV types associated with invasive cervical cancers as well in anal and head&neck ,so its specific identification has to be considered

Methods

In the market there are a very large numbers the genotyping test and it is well known that the different genotyping test has some analytical difference in performance . In this contest it is important to underline that WHO HPV Proficiency Panel results show the genotyping tests have very good performance across the lab for the HPV 16 detection while the main differences between the tests are related to identification of other HPV types. Similarly HPV tests validated for screening which allow a partial genotyping have high performance in terms of reproducibility and specificity for HPV 16 genotyping.

Results

In a screening setting when hpv test is used as primary screening test a testing with separate HPV16 and HPV18 detection can provide an alternative triage methods than cytology triage as well to follow women with abnormal screening results who are negative at colposcopy/biopsy and to predict the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN).

In women HPV vaccinated screening programs will change and the HPV test to be used has yet to be identified.

For anal neoplasia, genotyping, in general population ,is less important, because most of lesions are due to HPV 16, while it could be an important finding in HIV-positive subjects, but in this case it is appropriate a genotyping test able to identify all hpv types.

An increasing proportion of oropharyngeal squamous cell carcinomas (OPSCCs) is associated with human papillomavirus (HPV) type 16 infection. The identification of the presence of HPV infection

has clinical relevance, since there are important differences between tumors HPV-related and non. The first shows a tendency to grow in the oropharynx compared to other anatomical regions of the head and neck, an earlier age at diagnosis, and especially a more favorable prognosis and a better response to radio and chemotherapy treatments.

However survival studies have shown that the mere detection of HPV 16 DNA seems to be insufficient to identify the fraction actually related to viral infection. The simultaneous evaluation dell'overespressione the p16 protein or mRNA E6 / E7 of HPV allow proper identification of the fraction attributable to HPV.

Conclusion

The clinical utility of HPV 16 genotyping is clinical rilevante in different setting and not only for cervix but also for other HPV related cancer

SSim 03-04

POSITIONING THE ROLE AND VALUE OF THERAPEUTIC ANTI HPV 16/18 VACCINES - PRELIMINARY RESULTS OF GTL001 (PROCERVIX) IN CLINICAL TRIALS

P. Van Damme¹, M.C. Bissery², S. Olivier²

¹University of Antwerp (Belgium), ²Genticel (France)

Background / Objectives

Based on encouraging results from a phase 1 trial, Genticel initiated a randomized, double-blind, placebo-controlled study designed to demonstrate the efficacy of GTL001 (recombinant E7 HPV 16/18) adjuvanted with imiquimod in inducing viral clearance at one year post vaccination. Sustained clearance will be assessed two years after vaccination. The study enrolled women HPV 16 and/or 18 positive, with normal or ASCUS/LSIL cytology results. All women underwent a colposcopy at baseline with biopsy of any visible lesion to exclude patients with CIN2+ histology.

Methods

A total of 2371 women unaware of their HPV status were pre-screened with HPV genotyping test and cytology, of which 85 qualified for inclusion and additional screening procedures (3.6%). In addition, 504 women previously known to be positive for high-risk HPV infection were directly screened. Out of these 589 screened subjects, 350 were screen failures, 239 (40%) were randomly assigned to treatment, and 236 received at least one dose of study drug.

Results

The most frequent reasons for screen failure were not having HPV 16/18 infection (60%), not having cervical cytology evaluation with a normal, ASCUS, or LSIL (15%), withdrawal consent (7.5%), and concomitant episode of high-grade lesion (7%).

The population enrolled was generally healthy, with mean age of 35.6 years. Seventy-eight (78) % of women were HPV 16 positive while 19% were HPV 18 positive and 3% were positive for both HPV 16 and 18. A majority of subjects had abnormal cytology at baseline (172 ASCUS/LSIL, 73% versus 64 NILM, 27%). Seventy seven (77) subjects had biopsy at screening due to visible lesion(s) at colposcopy, of which 22 had CIN1 results. There were no significant differences between groups for demographic or baseline characteristics.

Three subjects did not receive the second injection of GTL001 because of severe local reactions following the first injection but remained in the study. Two subjects withdrew from the study during the vaccination period, one for non-compliance with study procedures and one for adverse event of pharyngitis/tonsillitis. Three subjects withdrew from the study during the follow-up period (2 subjects were lost to follow-up and one withdrew consent for reason independent of the study). A total of 231 subjects (97%) reached the 12-month visit and were evaluable for viral clearance at one year.

Conclusion

Results of the 12-month analysis will be presented.

ES-01

Understanding immune surveillance in cancer (Educational session)

P. Stern

University of Manchester (United kingdom)

Background / Objectives

Prospects for immunotherapy in HPV associated cancer:

Methods

This lecture will give an overview of the natural history of HPV infection and neoplasia and identify the key immune and viral factors.

Results

This will provide the background to understanding the prospects for utilising novel immunotherapies showing much promise for treatment of different cancers.

Conclusion

Immunotherapy for HPV associated disease is coming!

ES-02

The importance of inflammatory immune responses in HPV-induced carcinogenesis. (Educational session: Understanding immune surveillance in cancer)

S. Smola

Saarland University, Institute of Virology (Germany)

Background / Objectives

There is accumulating evidence that HPV-transformed keratinocytes actively contribute to the inflammatory microenvironment during cervical carcinogenesis promoting the progression from high-grade precursor lesions to invasive cancer.

Methods

This lecture will give an overview on inflammatory immune responses in cervical high-grade lesions and invasive cervical cancer.

Results

This lecture will provide detailed information on molecular mechanisms underlying inflammatory responses in HPV-driven carcinogenesis and potential consequences for targeted immunotherapies.

Conclusion

Understanding the immune microenvironment in HPV-associated carcinogenesis is important for the design of novel immunotherapies.

HN 01-01

Natural history of HPV in H&N region: where are we now?

S. Syrjänen

**Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine,
University of Turku, Turku (Finland)**

Background / Objectives

Approximately, 20% of head and neck squamous cell carcinomas (HNSCC) are associated with HPV, except for oropharyngeal SCC, of which nearly 60% are HPV-related. Although HPV has been settled as an etiological factor of a subgroup of these cancers, we still lack the natural history data showing the progression of HPV infection from asymptomatic lesions to intraepithelial neoplasia and cancer. However, meta-analysis covering case-control studies have shown that HPV is four times more likely to be present in oral potentially malignant lesions than in healthy oral mucosa (Syrjänen et al. 2012). The meta-analysis by Jayaprakash et al. (2011) showed no significant difference in HPV-16/18 prevalence rates (around 25%) between the dysplastic lesions and cancers in one hand, or between mild, moderate or severe dysplasia. They also reported that HPV was three times more likely to be present in male dysplastic lesions or SCC than in respective lesions in women.

Methods

So far, there are only two on-going follow-up (FU) studies on oral HPV infections: our Finnish Family HPV Study (FFHPV) on 331 families with six-year FU and HIM study on 23 men with oral HPV16 followed for 3-years. In the FFHPV totally 329 families were enrolled, comprising 329 mothers, 131 fathers and 331 newborns. The women were originally enrolled in the cohort at 36-weeks (minimum) of their index pregnancy and subsequently the parents and child to-come were followed up (FU) for 6 years.

Results

In our FFHPV study, point prevalence of oral HPV varied from 15% to 24% and 15% to 31% in women and men with 18 and 17 HPV genotypes, respectively. Most of the persisting oral infections in males were caused by HPV16. Smoking increased while previous genital warts decreased oral HR-HPV persistence. Altogether, 71.6% of the men cleared their infection. In women, HPV16 and HPV6 were the two most common genotypes and these were also the most likely to persist. Use of oral contraceptives and a second pregnancy protected against oral HPV persistence. Increased clearance was related with older age and a history of atopic reactions, whereas previous sexually transmitted disease and new pregnancy were associated with decreased clearance. The protective factors for

incident oral HPV-species 7/9 infections were 1) new pregnancy during FU, and 2) having the same sexual partner during FU. Persistent oral HPV16 infections in both genders are clearly associated with mixed or integrated physical state of the virus, while most of the cleared HPV16 infections are of episomal state.

Conclusion

Oral HPV infection is common in adults. HPV integration is important for HPV persistence and represents an early event in oral carcinogenesis.

References

Jayaprakash V et al. Human papillomavirus types 16 and 18 in epithelial dysplasia of oral cavity and oropharynx: a meta-analysis, 1985-2010. *Oral Oncol.* 2011 Nov;47(11):1048-54.

Syrjänen S et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Dis.* 2011 Apr;17 Suppl 1:58-72.

HN 01-02

HPV related and unrelated OPC: genomical differences

R. Brakenhoff

Tumor Biology section of Dept Otolaryngology-Head and Neck Surgery, VUmc Amsterdam (Netherlands)

Background / Objectives

Head and neck squamous cell carcinoma (HNSCC) develops in the mucosal linings of the upper aerodigestive tract and contributes to approximately 5% of all cancers in the Western world. Tumors develop either by exogenous carcinogen exposure (smoking, alcohol drinking) or human papillomavirus (HPV) infection. Particularly the squamous cell carcinomas in the oropharynx (OPSCC) encompass a high proportion of HPV-positive (HPV+ve) cases. The incidence of OPSCC is rising, which is attributed to HPV. Every year approximately 100,000 patients suffer from HPV-attributable OPSCC worldwide. HPV+ve and HPV-negative (HPV-ve) OPSCC are considered different disease entities. HPV+ve tumors have a much more favorable prognosis than HPV-ve tumors, and various risk models indicate that HPV status is the major predictor of prognosis in OPSCC. In 2004, it was shown for the first time that there are major genetic differences between HPV+ve and HPV-ve OPSCC. Subsequently a plethora of molecular studies revealed that besides major genetic differences, HPV+ve and HPV-ve OPSCC differ on the level of expression, microRNA profiles, and epigenetic profiles. In this presentation an overview of the molecular differences between HPV+ve and HPV-ve tumors will be presented, and the link to clinical behavior highlighted.

Methods

review

Conclusion

HPV+ve and HPV-negative (HPV-ve) OPSCC are considered different disease entities. HPV+ve tumors have a much more favorable prognosis than HPV-ve tumors, most likely as a consequence of major differences at the molecular level.

HN 01-03

Increased Incidence of Oropharynx Cancer Among the Elderly: An HPV-Associated Trend

Z. Zumsteg¹, G. Cook-Wiens¹, E. Yoshida¹, S. Shiao¹, N. Lee², A. Mita¹, C. Jeon¹, M. Goodman¹, A. Ho¹

¹Cedars Sinai Medical Center (United States of America), ²Memorial Sloan Kettering Medical Center (United States of America)

Background / Objectives

An increasing incidence of HPV-related oropharyngeal cancer has been reported predominantly among younger patients. Our objective was to update these results using United States cancer registry data with an emphasis on age-specific secular trends.

Methods

Data from 18 Surveillance, Epidemiology, and End results (SEER) program registries (2000-2012) were queried to compare secular changes in age-adjusted and age-specific incidence, and survival trends in oropharyngeal squamous cell carcinoma (OPSCC) with selected tobacco-related cancers (larynx, oral cavity, hypopharynx, lung) and HPV-related cancer (anus), by age, sex, and race-ethnicity.

Results

In total, 40,264 patients with OPSCC were included in this study. Significant increases in the age-adjusted incidence of OPSCC were observed during the study period for both younger adults aged 45 to 64 and elderly patients aged 65 or older, with similar annual percentage changes observed in both groups. These changes were driven predominantly by cancers in white men. Concomitantly, the incidence of tobacco-associated head and neck cancers and lung cancers decreased or were stable across these age groups, whereas the incidence of anal cancer markedly increased. Further, improved overall and cause specific survival over time were also observed for both younger and elderly patients with OPSCC. However, despite these improved relative outcomes, absolute cause specific survival remained worse for those over 65 in comparison to younger patients.

Conclusion

The incidence of OPSCC is increasing among elderly patients in the United States, likely driven by HPV-associated malignancies. Given the unique challenges related to treatment of elderly patients, their limited enrollment in clinical trials, and the aging United States population, clinical studies investigating improved therapeutic strategies for elderly patients with HPV-positive OPSCC are imperative.

HN 01-05

EPITHELIAL TO MESENCHYMAL TRANSITION AND HPV INFECTION IN SQUAMOUS CELL OROPHARYNGEAL CARCINOMAS: THE PAPILOPHAR STUDY

P. Birembaut

INSERM UMR-S 903 CHU Reims (France)

Background / Objectives

Human Papillomavirus (HPV) infection, particularly HPV16, is now recognized as a major etiological factor of carcinogenesis in oropharyngeal squamous cell carcinomas (OPSSC). HPV-related OPSSC respond better to treatments and generally have a significantly favorable survival outcome. By contrast, epithelial to mesenchymal transition (EMT) implicated in tumor invasion, is a hallmark of a poor prognosis in numerous carcinomas.

Our objective was to study the relationship of EMT markers with HPV infection and survival outcomes in a prospective study of a cohort of 302 well clinically characterized patients with OPSSC.

Methods

EMT was investigated by the immunohistochemical detection of E-cadherin, beta-catenin and vimentin in OPSSC. Considering vimentin as the best marker of EMT, EMT was graded according to vimentin scoring expression as follows: 0 = no EMT for vimentin score from 0 to 10%, 1 = mild EMT for vimentin score between 11 and 25%, 2 = overt EMT for vimentin score above 25%. HPV infection was identified by DNA and oncogenic proteins E6/E7 mRNA detection and p16ink4a immunohistochemical expression.

Results

Among the 302 OPSSC, 25.8% were HPV positive, 12% had mild EMT (and 20.6% overt EMT. In multivariate analysis, HPV positive patients had better loco-regional disease free survival (HR=0.290 [0.122; 0.692], p=0.0053), overall survival (HR=0.228 [0.109; 0.477], p <0.0001), event free survival (HR=0.431 [0.256; 0.724], p= 0.0015) than HPV negative patients (HR=0.431[0.256; 0.724], p=0.0015). EMT was an independent prognostic factor of metastasis free (overt EMT/no EMT: HR=1.750 [1.157; 2.644], p=0.008) and event-free survival (overt EMT/no EMT HR=3.773 [1.446; 9.845], p=0.0066).

There was a trend for, but non-significant association of EMT with HPV negative carcinomas. This may be explained by a mixed population with associated different risk factors (HPV, tobacco and alcohol), which may interfere in the tumorigenic pathways of these carcinomas.

Conclusion

The detection of EMT in OPSSC represents a reliable approach in the prognosis and eventually the treatment of these cancers whatever their HPV status.

HN 02-01

RISK OF ORAL HPV TRANSMISSION

G. D'souza

Johns Hopkins (United States of America)

Background / Objectives

This talk will review what is known about risk factors for oral HPV infection. Performing oral sex is the primary risk factor for oral HPV infection, but many people with oral HPV and HPV-related oropharyngeal cancer have modest number of oral sex partners. Oral HPV prevalence incidence is much lower than that for genital HPV, although oral sexual behavior is common. It has been noted for many years that men have a higher incidence of oral HPV infection and HPV-related oropharyngeal cancer than women, but reasons for this difference were not understood.

Methods

Recent data will be reviewed which help to explain these gender differences in oral HPV infection.

Results

Differences in oral HPV infection are not explained by behavior (i.e. when you compare men and women who both perform oral sex – with a similar number of people – men are still more likely to acquire an incident oral HPV infection than women. The more oral sex partners a man has, the higher their risk of oral HPV infection. In contrast, performing oral sex was not a risk factor for oral HPV infection in women. Instead, women with more vaginal sexual partners actually had a lower chance of getting an oral HPV infection.

Conclusion

This data is consistent with the hypothesis that women may mount a stronger immune response to genital HPV infection than men do, and when they do women may become protected from subsequent oral HPV infection. These data suggests higher rates of oral HPV infection in men are, at least in part, because they are less likely to have a strong natural immune response from prior genital HPV infection.

HN 02-04

ASSOCIATION OF HPV SEROLOGICAL MARKERS WITH HNSCC

T. Waterboer

Infections and Cancer Epidemiology, Molecular Diagnostics of Oncogenic Infections Division, German Cancer Research Center (DKFZ) (Germany)

Background / Objectives

Antibodies to HPV16 are strongly associated with head and neck squamous cell carcinoma (HNSCC), particularly oropharyngeal cancer (OPC). The presentation will summarize published case-control and prospective cohort studies using multiplex serology.

Methods

Antibodies to HPV16 L1, E1, E2, E4, E6, and E7 proteins in serum or plasma samples were analyzed by multiplex serology, a high-throughput method based on fluorescent bead technology that allows investigating up to 2000 serum samples per day for antibodies to up to 100 antigens simultaneously.

Results

In multiple studies, antibodies to HPV16 E6 were shown to be the serological marker most strongly associated with HNSCC, particularly with OPC, and much less so with oral cavity cancer. Overall, HPV16 serological markers were not associated with laryngeal or esophageal tumors. The prevalence of HPV16 E6 antibodies in healthy controls was repeatedly shown to be in the range of 0.5%, i.e. the specificity of this biomarker exceeds 99%. Antibodies to other HPV16 serological markers, especially E1, E2 and E7, were also associated with OPC, albeit less strongly, based on higher prevalence among controls and/or lower prevalence in OPC cases.

Conclusion

HPV 16 serological markers, especially antibodies to E6, are strongly associated with OPC in both case-control and prospective cohort studies, and have been repeatedly shown to be highly specific biomarkers for detection and prediction of OPC.

HN 02-05

Should HPV oropharynx cancer have its own staging?

S.H. Huang, B. O'sullivan

Princess Margaret Cancer Centre / University of Toronto (Canada)

Background / Objectives

Although tumor HPV status is often regarded as a prognostic biomarker due to dramatic differences in outcomes between HPV-related (HPV+) and unrelated (HPV-) oropharyngeal cancers (OPCs), its true role is as a diagnostic biomarker. In essence, HPV distinguishes two fundamentally different diseases affecting different populations with different clinical and outcome profiles. Although different in so many ways, they still use the same TNM classification (7th edition TNM, TNM-7ed). Emerging evidence suggests that TNM-7ed is inadequate to properly depict prognosis for HPV+ OPC patients. In addition, although current clinical trials address both diseases separately, clinicians and researchers are experiencing uncertainty in defining eligibility criteria for patient's selection. Consequently, opinions have converged on the principle that an HPV+ OPC specific staging system is urgently needed to properly depict the character and prognosis of this disease. The objective of this presentation is to assess the need for a new stage classification for HPV+ OPC.

Methods

A single-center cohort (a "Discovery cohort") formed the foundation to evaluate adequacy of TNM-7ed in HPV+ and HPV- OPC patients, suggesting a hypothesis that the current TNM-7ed is inadequate for HPV+ OPC; the study also assessed the feasibility of a proposed new stage classification for HPV+ OPC. The hypothesis was subsequently examined in a multi-centre setting ("Confirmatory Study") where the "Training Cohort" was chosen from the original centre and datasets from 6 additional institutions drawn from Europe and North America formed a "Validation Cohort". Finally, heterogeneity tests were performed to assess the performance of the new TNM classification.

Results

The multi-center "Confirmatory Study" based on the "Discovery Study" hypothesis proposed a new staging for HPV+ OPC. This used a Training-Validation design incorporating nearly 2000 HPV+ OPC treated from 7 institutions and two continents (North America and Europe). The major difference in the new stage classification for HPV+ OPC is that the N-categories resemble those used for nasopharyngeal cancer (NPC) excepting lower-neck lymph node involvement (a unique feature of the NPC classification). Heterogeneity tests confirmed that this hypothesis-based study upholds validity under various treatment protocols and jurisdictions.

Conclusion

Current TNM staging is inadequate for HPV+ OPC in predicting prognosis. A new stage classification for HPV+ OPC is needed. The newly proposed TNM classification permits a more appropriate depiction of the character and prognosis of this disease.

References

Huang SH, Xu W, Waldron J, Siu L, Shen X, Tong L, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM Stage and Prognostic Groups for Human Papillomavirus-Related Oropharyngeal Carcinomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015;33(8):836-45. Epub 2015/02/11.

O'Sullivan B, Huang SH, Su J, Garden A, Sturgis EM, Dahlstrom K, Lee N, Riaz N, Pei X, Koyfman SA, Adelstein D, Burkey BB, Friborg J, Kristensen CA, Gothelf AB, Hoebbers F, Kremer B, Speel E-J, Bowles DW, Raben D, Karam SD, Yu E, Xu W. A Proposal for UICC/AJCC Pre-treatment TNM Staging for HPV-related Oropharyngeal Cancer by the International Collaboration on Oropharyngeal Cancer Network for Staging (ICON-S): A Comparative Multi-Centre Cohort Study. *Lancet Oncol*. (accepted). Nov 27, 2015.

HN 03-01

Further studies on a model for predicting individual clinical outcome in patients with human papillomavirus (HPV) positive tonsillar and base of tongue cancer

A. Näsman ¹, C. Bersani ¹, N. Tertipis ¹, L. Haegglom ¹, L. Sivars ¹, T. Ramqvist ¹, E. Muck-Wikland ², M. Mints ¹, T. Dalianis ¹

¹Depts. of Oncology-Pathology, Karolinska Institutet, Stockholm (Sweden),

²Depts. CLINTEC, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Oropharyngeal cancer (OSCC) is increasing rapidly in incidence due to an increased incidence of human papillomavirus (HPV) positive tonsillar (TSCC) and tongue base (BOTSCC) squamous cell carcinoma. In addition, HPV positive TSCC and BOTSCC, but not other HPV positive OSCC, have been associated with a favorable clinical outcome. Despite the, in general, favorable outcome, all HPV positive TSCC and BOTSCC patients are today treated according to the same treatment protocols. We have previously identified prognostic markers e.g. high CD8+ tumour infiltrating lymphocytes (TILs) and low HLA class I expression, high LRIG1 etc, that with a high sensitivity identify HPV positive TSCC and BOTSCC patients with a favorable outcome, suggesting that a subset of these patients may benefit from a tapered treatment regime with maintained survival. However, the specificity is still low. Here the aim was to evaluate and to combine clinical and molecular markers into an algorithm for predicting outcome for individual patients with HPV DNA/p16(INK4a) positive TSCC and BOTSCC.

Methods

Previously 315 patients (197 as a training cohort and 118 as a validation cohort) treated curatively 2000-2011, with HPV DNA/p16INK4a positive tumors examined for HLA class I, CD8 tumor infiltrating lymphocytes (TILs) and other markers, were included in an L1-regularized logistic regression to evaluate the effect of the biomarker and clinical data, on 3-year risk of death or relapse. Presently, we are evaluating the samples for the presence of HPV E2 mRNA expression and hot spot mutations at different locations.

Results

Preliminary data show on a pilot cohort of 117 patients, that the combination of high CD8+ TIL numbers and presence of HPV16 E2 mRNA is an excellent combination. The experimental data will

shortly be completed in additional tumour samples with the aim to be included into the model for individual evaluation together with the other markers.

Conclusion

High CD8+ TIL numbers and presence of HPV16 E2 mRNA in HPV positive is the pilot study an excellent combination.

HN 03-02

PROMISE OF EARLY DETECTION OF HPV-OPC

T. Waterboer

Infections and Cancer Epidemiology, Molecular Diagnostics of Oncogenic Infections Division, German Cancer Research Center (DKFZ) (Germany)

Background / Objectives

Antibodies to HPV16, particularly the E6 oncoprotein, were strongly associated with HPV-driven oropharyngeal cancer (HPV-OPC) in two cross-sectional case series. Also, HPV16 E6 antibodies were shown to precede tumor diagnosis by more than 10 years in prospective cohort studies.

Methods

Antibodies to HPV16 L1, E1, E2, E4, E6, and E7 proteins in serum or plasma samples from two case series collected in Europe and the US, and two prospective studies, the European Prospective Investigation into Cancer and Nutrition (EPIC), and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), were analyzed by multiplex serology. Tumor HPV status was determined by HPV in-situ hybridization (ISH), HPV DNA detection, HPV RNA patterns (E6*^I, E1^{E4} and E1^C), and p16 immunohistochemistry (IHC).

Results

Antibodies to HPV16 E6 were shown to be almost exclusively present in cases with HPV-driven OPC, yielding both sensitivity and specificity exceeding 95%. At the same time, odds ratios for OPC prediction on average >6 years prior to diagnosis were >150.

Conclusion

HPV 16 serological markers, especially antibodies to E6, are strongly associated with OPC more than 10 years prior to clinical tumor diagnosis. To date, the trigger for seroconversion (e.g., infection of the tonsils or base of tongue, malignant transformation of tonsillar crypt epithelium, yet to be described early OPC lesions) is not understood, and the clinical implications of early HPV-OPC detection are under debate.

HN 04-01

PEMBROLIZUMAB IN HEAD AND NECK CANCER: PHASE 1 TRIAL RESULTS

F. Jin

Merck & Co., Inc. (United states minor outlying islands)

Background / Objectives

Patients (pts) with recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC) have limited treatment options. The programmed death (PD)-1 pathway is a key mechanism by which tumors evade immune surveillance. Approximately 60% of oropharynx cancers are associated with HPV infection. It is hypothesized that virally induced tumors may have enhanced immunogenicity and PD-L1 expression. Pembrolizumab (pembro), an anti-PD-1 antibody, blocks interaction between PD-1 and PD-L1. This presentation will summarize the current phase 1b data for pembro in R/M HNSCC.

Methods

KEYNOTE-012 (NCT01848834) is a phase 1b study evaluating the efficacy and safety of pembro in pts with advanced solid tumors, including 2 R/M HNSCC cohorts. The initial (I) HNSCC cohort enrolled PD-L1⁺ pts; these pts received pembro 10 mg/kg Q2W. Additional HNSCC pts were enrolled, regardless of PD-L1 status, in an expansion (E) cohort to further explore pembro in a different dosing regimen (200 mg Q3W). HPV status was collected for all HNSCC pts. Imaging was performed every 8 wk to assess treatment response. The primary endpoint was overall response rate (ORR; RECIST v1.1), and a key secondary endpoint was response among HPV⁺ pts.

Results

In total, 192 R/M HNSCC pts received pembro during this phase 1b trial (I=60 pts; E=132 pts). 32 pts were still on treatment at the data cutoff (Sept 1, 2015). The majority of pts (84%) received prior therapies for recurrent disease. ORR (confirmed) was 18% (95% CI, 13-24). 7 pts had a complete response; 27 pts had a partial response. By Sept 1, 2015, median duration of response was not yet reached (range, 2+ to 22+ mo); median follow-up duration for responders was 13 mo (range, 8-24). Responses were ongoing in 22 (76%) pts. ORR was 22% (95% CI, 13-34) in HPV⁺ pts and 16% (95% CI, 10-23) in HPV⁻ pts. Median OS was 9 mo (95% CI, 7-11); 6-mo PFS rate was 25%. Treatment-related AEs (TRAEs) occurred in 64% of pts; 12% of pts had a grade 3-4 TRAE. No pts died because of a TRAE.

Conclusion

The robust antitumor activity observed during this phase 1b study suggests pembro at either dose tested (200 mg Q3W or 10 mg/kg Q2W) is an active treatment for R/M HNSCC regardless of HPV status. Ongoing studies to assess the clinical benefit of pembro in HPV⁺ and HPV⁻ R/M HNSCC include KEYNOTE-055 (NCT02255097), a phase 2 trial of pembro after progression on platinum and cetuximab; KEYNOTE-040 (NCT02252042), a phase 3 trial of pembro vs standard of care (SOC) after platinum failure; and KEYNOTE-048 (NCT02358031), a phase 3 trial of first-line pembro or pembro+SOC vs SOC. Study outcomes may enable treatment optimization in pts with HPV⁺ and HPV⁻ R/M HNSCC.

HN 04-02

Intratumoral IL12 gene electro-transfer therapy for HNSCC

R. Pierce¹, **M. Dwyer**¹, **A. Mukhopadhyay**¹, **J. Haque**¹, **J. Campbell**¹, **C. Twitty**¹, **M. Le**¹, **S. Pai**², **C. Heaton**³, **C. Hultman**³, **T. Seiwert**⁴, **A. Algazi**³

¹OncoSec Medical Inc. (United States of America), ²Massachusetts General Hospital (United States of America), ³University of California San Francisco (United States of America), ⁴University of Chicago (United States of America)

Background / Objectives

Anti-PD1 blockade results in durable responses across a wide range of solid tumor types, including squamous cell carcinoma of the head and neck (HNSCC). The presence of PD1+ ‘partially exhausted’ CD8 T cells adjacent to PDL1+ cells within the tumor correlates with response to anti-PD1 blockade. This phenotype, often referred to as ‘adaptive immune resistance’, is thought to reflect compensatory upregulation of PDL1 due to IFN γ produced by CD8 T cells upon antigen binding. In contrast to high TIL tumors, which have a high probability of response to PD1 inhibition, the major pattern of PD1 nonresponsive tumors is a lack of these ‘exhausted’ TILs. A high prevalence of poorly immunogenic tumors represents a significant unmet medical need in immuno-oncology, in general, including in HNSCC. To convert PD1 nonresponders into responders, effective approaches are needed to enhance tumor immunogenicity and generate tumor-associated antigen (TAA)-specific TILs. IL-12 is a potent proinflammatory cytokine critical in driving anti-tumor Th1 immune responses.

Methods

We hypothesize that intratumoral electroporation of plasmid DNA-encoded IL-12 (IT-pIL12-EP) can lead to immunogenic cell death and release of TAAs, including neo-antigens, in the appropriate proinflammatory context to achieve an effective “in situ vaccination”. We developed a contralateral syngeneic mouse model, wherein tumor cells are implanted in the subcutis on the left and right flanks, allowing analysis of both treated and untreated tumors.

Results

Initial studies utilized the low TIL, PD1 refractory B16.F10 syngeneic mouse tumor model, stably transfected with the experimental neo-antigen ovalbumin (B16-OVA). In this model, IT-pIL12-EP leads to necrosis, a pleomorphic inflammatory infiltrate, generation of a proinflammatory gene signature, and complete regression of treated tumors (>95%). Growth of untreated distant tumors is significantly inhibited and correlates with expansion of specific anti-OVA (SIINFEKL+) CD8 T cells in

the spleen and in TILs. IT-pIL12-EP is now being tested in the E6/E7 (HPV-16) expressing TC1 mouse model.

Conclusion

Two Phase 2 trials with IT-pIL12-EP in Merkel cell carcinoma and melanoma have been completed. IT-pIL12-EP leads to sustained increases in intralesional IL-12 levels (tissue ELISA), increased TILs (IHC and Nanostring™-based gene expression analysis), abscopal effects, and objective clinical responses. To date, IT-pIL12-EP is safe and well-tolerated. Based on these data, a multicenter Phase 2 study of IT-pIL12-EP in treatment refractory metastatic and unresectable HNSCC was initiated. Interim clinical data and immunological correlates will be discussed.

HN 04-03

Strategies to Improve Response Rates in Recurrent and/or Metastatic Head and Neck Cancer Patients Who Have Progressed on Anti-PD-1, Anti-PD-L1, or Anti-CTLA-4 Monotherapy

S. Pai¹, **R. Haddad**², **N. Chau**², **J. Clark**¹, **A. O'Neill**², **P. Hammerman**², **L. Wirth**¹

¹Massachusetts General Hospital (United States of America), ²Dana Farber Cancer Institute (United States of America)

Background / Objectives

Human papillomavirus type 16 (HPV-16) actively recruits DNA methyltransferases (DNMTs) and/or histone deacetylases (HDACs) via the viral oncoprotein, E7, to strategically regulate viral gene expression during the viral life cycle in order to evade immune recognition and clearance by the host. We evaluated the role of epigenetic alterations in HPV-associated head and neck squamous cell carcinomas (HPV-HNSCCs), and mapped all potential DNA methylation sites within the HPV-16 epigenome in 22 patients with HPV-HNSCC. We identified 110 CpG sites within the HPV-16 viral methylome and reported that 45% (10 of the 22) of advanced stage HNSCCs were methylated in greater than 50% of the viral DNA CpG sites, with methylation clustered in the E1, E5, L1, and L2 genes (1). This finding suggests that the majority of the HPV viral proteins are not being expressed in the cancer cell. Furthermore, similar to HPV-associated cervical lesions, we found that as the number of viral DNA copies increased within a cancer cell the extent of viral DNA methylation also increased, which supports the hypothesis that there is active transcriptional suppression of the majority of the copies of the viral genome and, thus, expression of the immunogenic HPV viral antigens. The silencing of the redundant copies may be advantageous and even critical for clonal selection during carcinogenesis and may also facilitate immune evasion by the virus through decreased expression of immunogenic viral antigens. Another mechanism of immune evasion utilized by HPV and reported by our group was the activation of the Programmed Death-1 (PD-1): Programmed Death Ligand 1 (PD-L1) immune checkpoint pathway. Our findings supported a model in which the PD-1:PD-L1 immune checkpoint pathway becomes induced as an adaptive immune resistance mechanism of tumor against host. Preliminary results from a multi-institutional phase I study (www.clinicaltrials.gov; NCT01848834) demonstrated a 20% objective response rate in HPV-HNSCC patients with locally recurrent and/or metastatic disease treated with anti-PD-1 monotherapy. We hypothesize that head and neck cancer patients who failed anti-PD-1 monotherapy most likely failed due to a paucity of CD8+ T cells present within the tumor microenvironment. Thus, we are exploring whether administration of demethylating agents can re-activate the transcriptionally silent viral genomes and increase the expression of immunogenic HPV

antigens to increase the frequency of infiltrating cytotoxic CD8 + T cells and, thus, convert immune checkpoint blockade failures to responders.

References

1.Park IS et al, Cancer Prev Res 2011; 4(2):207-17

HN 04-04

IMMUNE BIOMARKERS IN RECURRENT HEAD AND NECK SQUAMOUS CELL CARCINOMA

D. Clayburgh

Oregon Health and Science University (United States of America)

Background / Objectives

Immune evasion is a critical process in carcinogenesis. Downregulation of host defense mechanisms and enhanced regulatory and tolerance pathways allow for tumors to grow unchecked by the immune system. In head and neck squamous cell carcinoma (HNSCC), there is growing interest in the use of immunomodulatory therapy to treat disease, particularly in the recurrent or metastatic setting. However, there is a relative paucity of data regarding the immunophenotype of recurrent HNSCC or immune biomarkers that may be associated with increased survival. Variability in the tumor immune microenvironment and immune markers may be a critical factor in determining tumor response to treatment, particularly in regards to newer immunomodulatory therapies. Thus, a multi-institutional collaboration has been established to analyze the association of immune biomarkers with patient outcomes and to understand the changes that occur in the tumor immune microenvironment after disease recurrence.

Methods

A collaborative project across multiple institutions in the United States was initiated to pool patients with recurrent HNSCC between 2010-2015. Patients were eligible for inclusion if they underwent radiation or chemoradiation for HNSCC, had recurrence of this tumor after treatment, and had pathologic specimens available for examination both before and after treatment. Demographic, tumor staging, treatment details, and survival outcomes were collected on all patients. Immunohistochemical analysis of tumor immune biomarker expression was performed, and correlated to outcome data.

Results

Patient identification and specimen collection is currently ongoing. Preliminary results indicate that changes in immune biomarker such PD-L1 within the tumor microenvironment are observed in the recurrent HNSCC setting and may correlate with survival.

Conclusion

Changes in the tumor immune microenvironment may be a key determinant of HNSCC tumor recurrence and patient outcomes in the recurrent setting. Characterization of immune biomarkers in recurrent HNSCC may provide insight into the role of the immune system in regulating tumor growth. Furthermore, these data may aid in selecting patients most likely to benefit from various immunomodulatory therapies, and may point the way to new therapeutic targets.

HN 05-01

THE ROLE OF COX-2/PGE₂ IN RECURRENT RESPIRATORY PAPILLOMATOSIS (RRP)

B. Steinberg¹, R. Wu¹, A. Lucs¹, A. Abramson², J. Devoti², V. Bonagura²

¹The Feinstein Institute for Medical Research, Northwell Health (United States of America), ²Long Island Jewish Medical Center, Northwell Health (United States of America)

Background / Objectives

Recurrent respiratory papillomas (RRP) are tumors of the respiratory airway, caused by HPVs 6/11. The disease is managed by surgery, but papillomas frequently recur due to activation of latent infection. Patients have an impaired immune response to HPV infection. Patients with RRP constitutively express COX-2 and its product PGE₂ in the airway epithelium. Here we review the role of PGE₂ in the pathobiology of RRP.

Methods

The role of PGE₂ was examined in primary cultured cells, a rabbit model system and *in vivo* in RRP patients. Papilloma cells, cultured in serum-free medium, were treated with PGE₂ or celecoxib, a selective COX-2 inhibitor, and analyzed for proliferation, apoptosis, and HPV mRNA expression and for activation of specific signaling pathways. Immature Langerhans cells (LCs) were generated from blood monocytes of RRP patients and controls, and exposed to varying concentrations of the cytokine IL-36 γ with/without added PGE₂. Expression of proinflammatory cytokine/chemokine expression was quantified by qPCR. Rabbits were infected with cottontail rabbit papilloma virus (CRPV) to establish a latent infection, treated with oral celecoxib or carrier, and treated with UV light to activate latency, which was measured by PCR. In a clinical trial, RRP patients were treated with celecoxib or placebo, the persistence of HPV latency was determined by PCR, and clinical responses were measured by rate of growth of recurrent papillomas.

Results

PGE₂ increases papilloma cell proliferation while celecoxib reduces proliferation and increases apoptosis. PGE₂ also increases HPV E6 and E7 transcription levels, through stimulation of the receptor EP4. Celecoxib inhibits activation of latent infection in the CRPV model. RRP patients' LCs were less responsive to IL-36 γ than controls, and PGE₂ strongly suppressed IL-36 γ -stimulated

expression of select cytokines and chemokines. In the clinical trial, 36% of patients showed sustained clinical improvement, and 13% were in remission at the end of the study, but response was not significantly different from the placebo group. Overall prevalence of latency was 50% in responders, the same value we had previously determined for patients with active disease.

Conclusion

COX-2/PGE₂ plays a major role in the pathophysiology of RRP. We propose that the therapeutic effects of celecoxib treatment in vivo in a subset of patients is due to suppressing latent activation, slowing papilloma growth and enhancing the local immune response.

HN 05-02

Epidemiology of recurrent respiratory papillomatosis

F. Buchinsky

Allegheny General Hospital, Pittsburgh, PA USA (United States of America)

Background / Objectives

Clinically significant RRP is defined as 1) a history of symptomatic breathing, swallowing, and/or voice problems; 2) the presence of wart-like lesions in the upper aerodigestive tract; and 3) histopathology demonstrating pedunculated masses with finger-like projections of nonkeratinized stratified squamous epithelium supported by a core of highly vascularized connective tissue stroma.

Methods

A brief review of the clinical epidemiology and disease course will be presented.

Results

The disease is rare; the incidence is approximately 1 per 100000 per year. RRP is diagnosed in both children and in adults and the disease course is variable. In the overwhelming majority of cases, the disease is caused by only two types of vaccine-preventable HPV: 6 or 11. While HPV (even if limited to type 6 and 11) is relatively common it is unknown why so few people develop RRP. The vast majority of the population either clear the virus or maintain subclinical colonization. Affected individuals often experience a heavy disease burden, undergoing many operations, and diminished quality of life due to chronic hoarseness which is often permanent.

The incidence of RRP for children born to mothers with condyloma acuminata is greatly increased in relative terms even if not in absolute terms. RRP is relatively more common among first-born children, born by vaginal delivery to a young mother (< 20 y). For adult-onset RRP, the risk factors are different: male gender and increased number of lifetime sexual partners.

Disease course in children can be very aggressive with early age of onset being associated with a more aggressive course. Regardless of age, person-to-person variability overwhelms our ability to predict a child's clinical course. Most children experience a decrease in disease aggressiveness until going into remission a mean of 4 years after diagnosis. Adults generally experience a more indolent course and there is an association between HPV 11 and a more aggressive course. Malignant transformation and pulmonary involvement are life-threatening complications although uncommon.

Conclusion

RRP is a rare but potentially devastating disease caused by two “low risk” HPV types better known for causing genital warts. The epidemiologic risk factors are well described and conceivably are markers of HPV exposure by two different predominant mechanisms: vertical transmission for juvenile-onset and sexual transmission for adult-onset.

HN 05-05

Management of Pediatric RRP--Update 2016

C. Derkay

**Eastern Virginia Medical School/Children's Hospital of the King's Daughters
(United States of America)**

Background / Objectives

Recurrent respiratory papillomas can be a devastating consequence of HPV infection in the upper aerodigestive tract. Surgical treatments have been more paliative than curative. This talk will review the current state-of-the-art in terms of surgical and adjuvant medical therapies in children and adults.

Methods

A comprehensive review of the current literature in combination with personal experiences in management of difficult cases.

Results

Results from case series and expert opinions will be presented with respect to the use of Cidofovir, Avastin, Celebrex and interferons. Ongoing trials will also be discussed.

Conclusion

The search for a "cure" continues to treat this vexing disease. The greatest optimism lies in prevention through vaccination.

References

Derkay CS, Wiatrak B. Recurrent Respiratory Papillomatosis: A Review. *Laryngoscope*. 2008; 118: 1236–1247.

Schraff S, Derkay CS, Burke B, Lawson L. American Society of Pediatric Otolaryngology members' experience with recurrent respiratory papillomatosis and the use of adjuvant therapy. *Arch Otolaryngol Head Neck Surg*. 2004; 130: 1039-1042

Chadha NK, James A. Adjuvant antiviral therapy for recurrent respiratory papillomatosis. Cochrane Database of Systematic Reviews. 2010; (1):CD005053.

HN 06-02

Transoral Surgical Resection Followed by Low-dose or Standard-dose IMRT in Resectable p16+ Locally Advanced Oropharynx Cancer

T. Thomas

Dana Farber Cancer Institute (United States of America)

Background / Objectives

Transoral Surgical Resection Followed by Low-dose or Standard-dose IMRT in Resectable p16+ Locally Advanced Oropharynx Cancer

Methods

Phase II Randomized Trial: ECOG 3311

Results

On going clinical trial

Conclusion

Ongoing clinical trial

HN 07-01

ROLE OF MUCOSAL ROUTE AND BLOCKADE OF CHECKPOINT INHIBITOR IN THE EFFICACY OF AN HPV THERAPEUTIC VACCINE FOR HEAD AND NECK CANCER

M. Nizard ¹, C. Badoual ¹, H. Roussel ¹, M. Diniz ², T. Tran ¹, T. Voron ¹, S. Karaki ¹, E. Dransart ³, L. Johannes ³, E. Tartour ¹

¹Hopital Européen Georges Pompidou. INSERM U970. Université Paris Descartes (France), ²Institute of Biomedical Sciences, University of São Paulo, Brazil (Brazil), ³Institut Curie, PSL* Research University, Endocytic Trafficking and Intracellular Delivery team (France)

Background / Objectives

Checkpoint inhibitors led to clinical successes mainly in tumors with pre-existing anti-tumor T cells. In a preclinical model of HPV-E6-E7 expressing tumor with no spontaneous T cell infiltration, we test whether the induction of a recruitment of CD8+T cells by a vaccine would improve the efficacy of a therapeutic HPV vaccine.

Head and neck cancers are located at mucosal sites. We had previously shown that mucosal immunization is correlated with a strong protection against orthotopic head and neck cancer in both prophylactic and therapeutic settings, while systemic immunization was not (Sandoval F Sci Transl Med 2013). However, the mechanisms related to this protection remain unaddressed

Methods

We used a tumor model (TC1) expressing the E6-E7 protein from HPV16 grafted on a subcutaneous site or in the tongue (mucosal head and neck orthotopic site).

The therapeutic HPV vaccine selected was composed of the B subunit of Shiga toxin – a delivery vector targeting dendritic cells – fused to the E7 protein from HPV 16 (STxB-E7).

This vaccine was administered by the subcutaneous (systemic) route or the intranasal (mucosal) route alone or combined with anti-PD-L1.

Results

We showed that when TC1 cells were grafted into mice, its growth was rapid and there was a poor infiltration with CD8+T cells. Administration of anti-PD-L1 mAb were inefficient to control the growth of this tumor. However, a synergy was observed when STxB-E7 was combined with anti-PD-L1. This synergy could be explained by the recruitment of intratumor CD8+T cells by the vaccine and the upregulation of PD-1 on these cells. In a second work, we tried to understand the mechanisms explaining the superiority of the intranasal route over the systemic route to control orthotopic head and neck cancers. Various experiments showed that resident memory CD8+T cells play a key role in the protection induced by the mucosal route of immunization. Finally, in human mucosal tumors, we confirmed the presence of Trm in situ and the improvement of survival in patients showing intratumoral Trm infiltration

Conclusion

Therapeutic vaccine may represent a rational combination with checkpoint blockade for tumors with no spontaneous T cell infiltration

Monitoring Trm may be used as a new surrogate biomarker of patient survival and may represent a new marker of vaccine efficacy for mucosal tumors.

References

Sandoval F et al Sci Transl Med 2013

Badoual C et al Cancer Res 2013

Nizard M et al Hum Vaccin Immunother 2014

Nizard M et al Clin Cancer Res 2015

HN 07-02

SUPERIOR PREDICTION OF RESPONSE TO THERAPY BY MEASUREMENT OF INTRATUMORAL HPV- SPECIFIC IMMUNITY

**M. Welters, L.A. Van Der Velden, V. Van Ham, I. Ehsan, R. Goedemans,
S. Santegoets, S. Van Der Burg**

Leiden University Medical Center (Netherlands)

Background / Objectives

The incidence of head and neck squamous cell carcinoma (HNSCC) is decreasing, while within this group the number of oropharyngeal SCC (OSCC) is rising, especially in young adults. The human papillomavirus type 16 (HPV16) is attributable for 45-90% of these OSCC, which interestingly results in a better response to therapy compared to HPV-negative OSCC. This improved clinical outcome is independent of nodal status, age, stage, tumor differentiation or gender. As previously HPV16-specific T-cell responses were measured in blood and tumor of HPV16+ OSCC, we hypothesized that these T-cells impact the tumor microenvironment and thereby support a better therapeutic efficacy.

Methods

Tumor sections of 87 OSCC patients were HPV typed by GP5+/6+ PCR and stained for p16 expression by immunohistochemistry. Freshly dispersed and cryopreserved tumor tissues were used for phenotypical analysis (CYTOF and flow cytometer) to determine the leukocyte infiltration composition. Moreover, the ex vivo HPV16-specific T-cell reactivity was determined in these samples.

Results

In our cohort, 57% of OSCC patients were HPV16+ and these patients showed the best overall survival ($p < 0.0001$, HR 6.9), as estimated by Kaplan-Meier curves and log-rank analysis. In 63% of these HPV16+ OSCC patients an intratumoral HPV16 E6/E7-specific T-cell reactivity was detected. Notably, no proliferative T-cell responses were observed in the HPV-negative OSCC patients. Within the HPV16+ OSCC patient group the presence of HPV16-specific immunity correlated with a better clinical outcome ($p = 0.0003$, HR 23.9). Also grouping of these HPV16+ OSCC patients according to their p16 expression, showed that p16+ patients are doing better ($p < 0.0001$, HR 11.8). As overexpression of p16 is directly associated with the expression of the viral oncoproteins E6 and E7, the association between p16 expression and intratumoral HPV16-specific T-cell reactivity ($p < 0.0001$) is in line with this. In vitro mechanistic studies revealed that OSCC cell lines promoted monocyte to

differentiate into tumor-promoting M2 macrophages, which can be prevented by cytokines secreted by HPV16-specific T cells. Importantly, the resident M2 macrophages could be redirected into better antigen-presenting cells in terms of phenotypically (induction of co-stimulatory molecules) and functionally (>IL-12 and <IL-10 production). Interestingly, the type 1 T-cell cytokines also interfered directly with proliferation and cell death of OSCC cells.

Conclusion

The intratumoral HPV16-specific T-cell responses, elicited by HPV16+ OSCC, is an important component for the clinical efficacy of standard therapy.

References

Funding: Dutch Cancer Society UL 2013-6146

HN 07-03

A NEW MUCOSAL ROUTE FOR THERAPEUTIC VACCINES AGAINST HEAD AND NECK SQUAMOUS CELL CARCINOMAS

R. Macedo¹, J. Rochefort¹, M. Guillot-Delost¹, K. Tanaka¹, A. Le Moignic¹, C. Noizat¹, C. Baillou¹, V. Mateo¹, A.F. Carpentier², E. Tartour³, C. Bertolus⁴, B. Bellier⁵, G. Lescaille⁶, F.M. Lemoine⁶

¹Sorbonne Universités, UPMC/Paris 06, UMR-S INSERM U1135, CNRS ERL 8255, Centre d'Immunologie et Maladies Infectieuses (CIMI-Paris); Paris, France (France), ²Université Paris 13; AP-HP, Hôpital Avicenne, Department of Neurology; Bobigny, France. (France), ³Paris Descartes/Paris 05, Sorbonne Paris Cité; INSERM U970, Paris-Cardiovascular Research Center (PARCC); AP-HP, Hôpital Européen Georges Pompidou, Service d'Immunologie Biologique; Paris, France. (France), ⁴Sorbonne Universités, UPMC Univ-Paris 06; AP-HP, Groupe hospitalier Pitié-Salpêtrière, Department of Maxillo-facial Surgery; Paris, France. (France), ⁵Sorbonne Universités, UPMC/Paris 06, UMR-S INSERM U959, CNRS, FRE 3632, Immunology-Immunopathology-Immunotherapy (I3); Paris, France (France), ⁶Sorbonne Universités, UPMC/Paris 06, UMR-S INSERM U1135, CNRS ERL 8255, Centre d'Immunologie et Maladies Infectieuses (CIMI-Paris); Paris, France. (France)

Background / Objectives

Despite current therapy, head and neck squamous cell cancers (HNSCCs) arising from various mucosal sites of the upper aero-digestive tract frequently relapse in a loco-regional manner and have a poor prognosis. Our objective was to validate an innovative mucosal route of vaccination using plasmid virus-like particles (pVLPs) in a pre-clinical orthotopic model of HNSCCs.

Methods

pVLP-E7, that are plasmid DNA encoding retroviral virus-like particles carrying a truncated E7 oncoprotein from HPV16 as antigen model, were used to vaccinate mice bearing pre-established TC-1 tumors implanted into the buccal mucosa. pVLP-E7 were combined with clinical grade TLR agonists (Imiquimod and CpG-ODN). In this pre-clinical orthotopic model, whose tumor microenvironment resembles to those of human HNSCCs, we tested different mucosal vaccination routes for their ability to elicit efficient immune and anti-tumoral responses.

Results

Mucosal intra-cheek (IC) vaccinations using pVLP-E7, comparatively to intradermic vaccinations (ID), gave rise to higher mobilization of mucosal (CD49a+) CD8+ specific effector T cells in both tumor draining lymph nodes and tumor microenvironment resulting in better anti-tumor effects and in a long-term protection against tumor rechallenge. In vivo CD8 depletion demonstrated that anti-tumoral effects were fully dependent upon the presence of CD8+ T cells.

Conclusion

Validation of IC mucosal vaccinations with pVLPs combined with adjuvants using a pre-clinical orthotopic model of HNSCC provide valuable pre-clinical data to rapidly envision the use of such therapeutic vaccines and to implement a clinical trial in patients with HNSCCs, inasmuch as vaccinal components and adjuvants can be easily obtained as clinical grade reagents.

HN 08-01

SLPI and Annexin A2 expression in non-neoplastic tonsillar tissue specimens in correlation to smoking habit

M. Hoffmann¹, A.S. Hoffmann², L. Getzin¹, B. Bögershausen², T. Görögh¹, M. Gebhardt³, R. Pries², E.S. Quabius¹

¹Dept. of ORL, H&N Surgery, Christian-Albrechts-University, Kiel (Germany),

²Dept. of ORL, H&N Surgery, University Clinic Schleswig-Holstein, Campus Lübeck (Germany), ³Instt. of Pathology, University Clinic Schleswig-Holstein, Campus Lübeck (Germany)

Background / Objectives

Retrospectively, we correlated SLPI- and Annexin A2-expression with the HPV-status in chronic tonsillitis (CT; n=118) or tonsillar hyperplasia (H; n=96) tissue specimens, respectively, to analyse in non-neoplastic tonsillar tissue the inverse correlation between SLPI-expression and HPV-infection that was previously shown in HNSCC. We hypothesise that smoking induced up-regulation of SLPI results in a reduced binding of HPV to Annexin A2, a known modulator of HPV-entry in to the cell.

Methods

SLPI and p16INK4A-protein expression was measured by immunohistochemistry (IHC) in all 214 specimens; SLPI and Annexin A2 (AnxA2) gene expression was measured by RT-qPCR in 138 cases; DNA was isolated from all specimens to determine HPV-status. Results were correlated with patients' smoking habit.

Results

In total 118 (55.1%) patients had CT (71 non-smokers; 47 smokers) and 94 (44.9%) had H (95 non-smokers; 1 smoker; 1 unknown). All samples were HPV-negative. p16INK4A-expression showed negative, weak, and moderate signals in 20 (9.3%), 156 (72.9%), and 38 (17.8%) cases, respectively. SLPI protein-expression showed negative, weak, and moderate signals in 163 (76.2%), 45 (21.0%), and 6 (2.8%) cases, respectively. The correlation between smoking and SLPI was overall $p=0.0001$. Gene-expression analysis (n=138) showed that smoking (n=48) resulted in 5.39-fold increased SLPI-expression together with a 4.49-fold increase in AnxA2. A significant positive correlation between AnxA2 and SLPI indicating a surplus of AnxA2 in relation to SLPI was only found in the tonsillar tissue of non-smokers.

Conclusion

The data demonstrate that smoking not only, as previously shown results in HNSCC in increased SLPI- and AnxA2-expression, but show that this is also the case in non-neoplastic tonsillar tissue. The observed surplus of AnxA2 in relation to SLPI only found in the tonsillar tissue of non-smokers indicates a higher probability of a successful HPV-infection of the tonsillar tissue of non-smokers, given the properties of AnxA2 to function as an infection-modulator. The data demonstrate that smoking not only, as previously shown results in HNSCC in increased SLPI- and AnxA2-expression, but show that this is also the case in non-neoplastic tonsillar tissue. The observed surplus of AnxA2 in relation to SLPI only found in the tonsillar tissue of non-smokers indicates a higher probability of a successful HPV-infection of the tonsillar tissue of non-smokers, given the properties of AnxA2 to function as an infection-modulator.

References

- Quabius ES et al., *Cancer Lett* 2015;357(1):339-45
- Quabius et al., *Int J Cancer* 2014;15;134(6):1323-34
- Hoffmann et al., *Oncol Rep.* 2013;29(5):1962-8

HN 08-02

Integration of human papillomavirus type 11 into FGFR3 gene and long non-protein coding RNA LINC00486 in a patient with sinonasal carcinoma

L. Hošnjak¹, B.J. Kocjan¹, T.M. Zorec¹, N. Gale², N. Zidar², P. Strojan³, M. Poljak¹

¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia (Slovenia), ²Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia (Slovenia), ³Department of Radiation Oncology, Institute of Oncology, Ljubljana, Slovenia (Slovenia)

Background / Objectives

Low-risk human papillomavirus types 6 and 11 (HPV6 and HPV11), etiological agents of genital warts and laryngeal papillomas, are rarely associated with human cancers. However, HPV6 and HPV11 may incidentally integrate into the human genome and cause malignant transformation by insertional inactivation of particular human oncogenes (1) or intergenic regulatory regions (2). The objective of the present study was to investigate further the molecular mechanism(s) of low-risk HPV-mediated carcinogenesis by studying a case of HPV11-positive sinonasal carcinoma.

Methods

Archival tissue specimen of the sinonasal carcinoma, HPV11-positive with INNO-LiPA HPV Genotyping Extra test (Innogenetics), was tested for the presence of HPV6/11 DNA, HPV6/11 E6/E7 mRNA and high-risk HPV E6/E7 mRNA by in-situ hybridization (ISH), using INFORM HPV II Family 6 Probe (B) (Roche Diagnostics), Probe-HPV11 and Probe-HPV HR7 (Advanced Cell Diagnostics), respectively. Immunohistochemical stainings for p16^{INK4A}, p53 and pRB proteins were performed using CINtec p16 Histology (Ventana Medical Systems), Monoclonal Mouse Anti-Human p53 Protein Clone DO-7 (Dako) and Rb Antibody (Santa Cruz Biotechnology), respectively. For next generation sequencing (NGS), 2 µg of extracted DNA were used to generate TruSeq Nano paired-end libraries and sequenced using Illumina NextSeq instrument.

Results

Using NGS complete HPV11 genome sequence (7,933-bp), showing 99.7% nucleotide similarity to the HPV11 reference sequence, was obtained from the sinonasal carcinoma. None of the identified nucleotide/amino acid changes in the LCR genomic region and E7, E1, E2 and E5a proteins clustered

within their functional domains. HPV6/11 DNA ISH showed nuclei harboring a single punctate signal, indicating HPV integration. HPV6/11 E6/E7 mRNA ISH revealed active expression of viral oncogenes, high-risk HPV E6/E7 mRNA ISH was negative, and p16^{INK4A}, p53 and pRB were highly expressed. NGS data analysis revealed that HPV11 was integrated in the chromosome 2 (2p22.3), within the Long Intergenic Non-protein Coding RNA 486 (LINC00486) region. Additionally, short parts of the HPV11 LCR were integrated in the chromosome 4 (4p16.3), within or in the close proximity of the FGFR3 gene.

Conclusion

HPV11 integration upstream of and within the FGFR3 gene and additionally in the LINC00486 region, and overexpression of p53 and pRB which are normally downregulated in high-risk HPV-induced malignant tumors, were detected in the sinonasal carcinoma. Alternations in expression and structural changes of the FGFR3 protein and long non-coding RNA complexes have been previously associated with some types of human cancers, thus our case warrants further research.

References

- (1) Huebbers CU, Preuss SF, Kolligs J, Vent J, Stenner M, Wieland U, Silling S, Drebber U, Speel EJ, Klussmann JP. Integration of HPV6 and downregulation of AKR1C3 expression mark malignant transformation in a patient with juvenile-onset laryngeal papillomatosis. *PLoS One* 2013;8(2):e57207.
- (2) Scheel A, Lin GC, McHugh JB, Komarck CM, Walline HM, Prince ME, Zacharek MA, Carey TE. Human papillomavirus infection and biomarkers in sinonasal inverted papillomas: clinical significance and molecular mechanisms. *Int Forum Allergy Rhinol* 2015;5(8):701-7.

HN 08-03

HUMAN PAPILLOMAVIRUS INFECTION AND HEAD AND NECK CANCERS IN MONTREAL, CANADA: RESULTS FROM THE HENCE LIFE CASE-CONTROL STUDY

C. Laprise¹, **S.A. Madathil**², **N.F. Schlecht**³, **D. Soulières**⁴, **P.F. Nguyen-Tan**⁵, **G. Castonguay**⁶, **P. Allison**⁶, **F. Coutlée**⁷, **M.C. Rousseau**², **E.L. Franco**⁸, **B. Nicolau**⁶

¹Department of Oncology, Division of Cancer Epidemiology, McGill University, Montreal; Oral Health and Society Division, McGill University, Montreal (Canada), ²Oral Health and Society Division, McGill University, Montreal; Epidemiology and Biostatistics Unit, INRS-Institut Armand-Frappier, Laval (Canada), ³Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York (United States of America), ⁴Department of Hemato-Oncology, Hôpital Notre-Dame du Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal (Canada), ⁵Department of Radiation Oncology, Hôpital Notre-Dame du Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal (Canada), ⁶Oral Health and Society Division, McGill University, Montreal (Canada), ⁷Department of Microbiology and Infectious Diseases, Hôpital Notre-Dame du Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal (Canada), ⁸Department of Oncology, Division of Cancer Epidemiology, McGill University, Montreal (Canada)

Background / Objectives

Human papillomavirus (HPV) is a strong risk factor for a subset of head and neck cancers (HNCs), primarily oropharynx cancers. However, the distribution of specific HPV genotypes and their association with risk of cancer in anatomical subsites needs to be better understood. Our objectives were to describe HPV prevalence and to estimate the extent with which HPV infection is associated with HNC risk overall and for oral cavity, larynx, and oropharynx cancers.

Methods

The Canadian site of the HeNCe Life study, an international hospital-based case-control study, recruited 389 incident HNC cases from four hospitals in the Montreal area. A total of 429 controls from outpatient clinics at the same hospitals as the cases were recruited and frequency-matched by age and sex. Data collected through interviews included socio-demographic, environmental and behavior information. Oral rinse and oral brush specimen, collected in both cases and controls, were

analyzed for HPV positivity and HPV genotyping. HPV status was categorized as either negative, HPV other than alpha-9 species, alpha-9 other than HPV16, or HPV16. Unconditional logistic regression were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between HPV infection and HNC, while adjusting for age, sex, number of educational years, and lifetime smoking and alcohol consumption.

Results

HPV DNA was detected in 41.9% of cases and 14.2% of controls. The prevalence of HPV infection in cases was 62.6% in the oropharynx, 26.5% in the larynx and 20.0% in the oral cavity. HPV16 was the most prevalent genotype detected among both cases (29.8%) and controls (2.3%); 48.1% of oropharynx cancer cases tested positive for HPV16. HPV infection was associated with an increased risk of HNC in both oral brush (OR=8.6; 95% CI 4.6-15.8) or oral rinse samples (OR=4.1; 95% CI 2.9-5.8). Similarly, associations were observed for oropharyngeal subsite cancers both in oral brush specimen (OR=18.4; 95% CI 9.7-34.7) and oral rinse (OR=9.1; 95% CI 5.9-13.8, oral rinse). HNC risk was moderately associated with HPVs alpha-9 other than HPV16 (OR=2.9; 95% CI, 1.2-7.1). HNC risk was strongly associated with HPV16, especially for oropharynx (OR=45.5; 95% CI, 22.3-91.9), relative to larynx (OR=3.1; 95% CI, 1.1-8.5) and oral cavity (OR=4.5; 95% CI, 1.4-14.1) sites.

Conclusion

As expected, HPV infection was associated with an increased HNC risk, especially HPV16 with oropharyngeal cancer. In our study, the role of HPV in HNC risk varies by HNC subsite and is related to HPV genotype.

HN 08-04

EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT) SIGNATURE IN HPV-POSITIVE AND HPV-NEGATIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

C. Mourareau¹, C. Boulagnon-Rombi², B. Nawrocki-Raby¹, P. Birembaut², M. Polette¹, C. Clavel¹, V. Dalstein¹

¹INSERM, UMRS 903, SFR CAP-Santé, URCA, CHU Maison Blanche, Reims, France (France), ²Laboratoire de Biopathologie, CHU Robert Debré, Reims, France (France)

Background / Objectives

Oropharyngeal squamous cell carcinoma (OSCC) presents heterogeneous clinical behavior and response to therapies. There is a need to improve the classification to better stratify patients and therapeutic options. HPV-positive OSCC subset is recognized to have generally a much more favorable prognosis. To the opposite, epithelial-to-mesenchymal transition (EMT), a key process associated with tumor progression and metastasis, is associated with poor prognosis. This process is known to increase treatment resistance in many cancers. It also could play a role in oropharyngeal carcinoma and could explain the clinical evolution.

The aim of this work was to characterize the EMT signature of HPV-positive and HPV-negative OSCC in a retrospective cohort.

Methods

A total of 40 patients diagnosed between 2004 and 2013 in the University Hospital of Reims and operated for OSCC were included in this study. Median age at diagnostic was 57 years [38-89]. FFPE specimens were evaluated for HPV status using HPV genotyping (Innolipa), E6E7 RT-qPCR, in situ hybridization for high-risk HPV-DNA and p16 immunohistochemistry. EMT marker expression was detected by immunohistochemistry, to evaluate expression loss of epithelial markers (E-cadherin and β -catenin) and expression gain of mesenchymal markers (vimentin and N-cadherin).

Results

Ten OSCC cases (25 %) were found to be HPV-positive. Twenty-nine cases (72,5 %) harbored one or more EMT markers. This seems to be more frequent in HPV-negative group (80 %) than in HPV-positive group (50 %) but this association was not statistically significant ($p=0,066$). As expected, HPV

status was associated with a better overall survival and relapse-free survival. In the opposite, EMT was associated with a worse overall survival and early relapse-free survival. This was also the case specifically in the HPV-negative group but could not be verified in the HPV-positive group, due to the small size of the group.

Conclusion

We draw the hypothesis that EMT and HPV status could be combined to define different prognostic outcomes: more favorable for EMT-/HPV+, less favorable for EMT+/HPV-. The understanding of the role of EMT in HPV-induced OSCC could help to select therapeutic options and particularly to properly select HPV-positive OSCC patients that could benefit from treatment de-intensification.

HN 08-05

META-ANALYSIS ON THE ACCURACY OF P16INK4A IMMUNOHISTOCHEMISTRY TO DIAGNOSE HPV-INDUCED OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS

E.S. Prigge¹, M. Arbyn², M. Von Knebel Doeberitz¹, M. Reuschenbach

¹Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), Heidelberg (Germany), ²Belgian Cancer Centre and Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, and University of Antwerp, Antwerp (Belgium)

Background / Objectives

Overexpression of the cell cycle protein p16^{INK4a} on immunohistochemistry (IHC) is widely applied as a surrogate marker of etiological HPV involvement in oropharyngeal squamous cell carcinomas (OPSCC). However, the accuracy of this marker to identify a transforming HPV infection in OPSCC varies considerably between different studies. We performed a meta-analysis on the accuracy of p16^{INK4a} IHC to identify HPV-transformed OPSCC. The diagnostic accuracy of p16^{INK4a} IHC was compared to that of other commonly applied HPV detection tests.

Methods

All available studies that analyzed HPV E6 and/or E7 oncogene transcription by an amplification-based method (gold standard to diagnose a transforming HPV infection) and p16^{INK4a} by IHC in OPSCC were included in the analysis. Pooled sensitivity and specificity of p16^{INK4a} IHC was computed and compared to the diagnostic accuracy of the comparator tests HPV DNA detection by PCR, HPV DNA detection by in situ-hybridization (ISH) and the test combination p16^{INK4a} IHC/HPV DNA detection by PCR.

Results

The inclusion criteria were fulfilled by 24 studies. The pooled sensitivity of p16^{INK4a} IHC, HPV DNA PCR, HPV DNA ISH and combined p16^{INK4a}/HPV DNA PCR was 0.94 (95% confidence interval (CI) 0.91-0.97), 0.97 (95% CI, 0.93-1.00), 0.85 (95% CI, 0.76-0.92) and 0.93 (95% CI, 0.87-0.97), respectively. The pooled specificities were 0.83 (95% CI, 0.78-0.88), 0.86 (95% CI, 0.77-0.93), 0.88

(95% CI, 0.78-0.96) and 0.96 (95% CI, 0.89-1.00), respectively. Relative sensitivity and specificity computation revealed that co-testing for p16^{INK4a} IHC and HPV DNA PCR was as sensitive but significantly more specific than both individual tests.

Conclusion

Our data demonstrate that p16^{INK4a} IHC when used as a single test is very sensitive and moderately specific to diagnose HPV-transformed OPSCC. Co-testing with p16^{INK4a} IHC and HPV DNA detection by PCR significantly increases the diagnostic specificity while maintaining high sensitivity compared to the individual tests. The combined testing is thus suggested as a meaningful testing strategy for the reliable diagnosis of HPV-transformed OPSCC.

HN 08-06

MiRNA-EXPRESSION IN TONSILLAR CARCINOMAS IN RELATION TO HPV-INFECTION AND EXPRESSION OF THE ANTILEUKOPROTEINASE SLPI

E.S. Quabius¹, I. Merz¹, T. Görögh¹, J. Hedderich², C. Röcken³, P. Ambrosch¹, M. Hoffmann¹

¹Department of Otorhinolaryngology, Head and Neck Surgery, Christian-Albrechts-University Kiel (Germany), ²Institute for Medical Informatics and Statistics, Christian-Albrechts-University Kiel (Germany), ³Institute for Pathology, Christian-Albrechts-University Kiel (Germany)

Background / Objectives

miRNAs are small non-coding mRNA binding nucleic acids which exhibit regulatory properties. It is known that their expression is significantly different between HPV-positive und –negative head and neck carcinomas. Previously, 36 miRNAs were described as being differentially expressed in these tumours [1]. So far, it was not analysed if this differential miRNA expression is relevant in the context of the known inverse correlation between HPV-status and expression of the antileukoprotease SLPI.

Methods

In 126 tonsillar carcinomas with known HPV-status (42.06% positive) and SLPI-protein expression levels, the 4 highest modulated miRNAs (miRNA 363, miRNA 21, miRNAs 31 und 193b*) [1] were analysed by RT-q-PCR and their correlation between HPV-status and SLPI-expression was determined. In silico, the SLPI sequence was screened for potential binding sites of the 36 differentially expressed miRNAs. Kaplan-Meier-Analysis was performed correlating HPV-status, SLPI- and miRNA-expression levels with overall- and progression free-survival.

Results

Results indicate a 12- and 6-fold up-regulation of miRNA 363 and miRNA 21, respectively, and a 5-fold down-regulation of miRNAs 31 und 193b* in HPV-positive versus HPV-negative tonsillar carcinomas. Interestingly, the most up-regulated miRNA, namely miRNA 363, is the only one without potential binding sites in the SLPI-mRNA, however, potential binding to HPV mRNAs E6 and E7 is possible. In addition miRNA 363 shows the highest expression levels in HPV-positive but SPLI-negative carcinomas. HPV-positivity, as well as low SLPI- and high miRNA 363-expression levels are correlated with significantly better overall and progression free survival (all $p < 0.05$).

Conclusion

Firstly, the results confirm the findings by Lajer and coworkers [1] with divergent expression levels in HPV-positive carcinomas and with miRNA 363 as the most up-regulated miRNA. The here, for the first time described, interplay between SLPI-expression and HPV-status on the one hand and the relation of miRNA 363 with HPV-positive but SPLI-negative carcinomas on the other hand, as well as the prognostic value of miRNA 363 suggests a role for this miRNA in the SLPI-modulated HPV-infection of tonsillar, but possibly also in all head and neck carcinomas.

References

[1] C.B. Lajer et al. (2012) Br J Cancer 106(9):1526-1534.

HN 08-07

BRANCHIOGENIC CARCINOMA WITH HIGH-RISK TYPE HUMAN PAPILLOMAVIRUS INFECTION

M. Suzuki, H. Maeda, T. Ikegami, H. Hirakawa, Z. Deng

Department of Otorhinolaryngology, Head and Neck Surgery, University of the Ryukyus (Japan)

Background / Objectives

Branchiogenic carcinoma (BC) appears as a mass lesion with a predominant cystic component. Since lymph node metastasis from oropharyngeal carcinoma has a cystic appearance, it is sometimes difficult to distinguish BC from nodal metastases from clinically silent oropharyngeal carcinoma. Factors related to the malignant transformation process in BC remain obscure.

Methods

A 56-year-old man had a right cervical mass that was diagnosed as squamous cell carcinoma by needle biopsy. The primary tumor could not be detected despite several imaging examinations, pan-endoscopy of the head and neck, esophagus, and stomach, and biopsies of the head and neck regions including bilateral tonsillectomy. HPV infection in this case was examined by conventional PCR, p16^{INK4a} immunohistochemistry, and in situ hybridization. In addition, the viral load of HPV was determined by quantitative real-time PCR.

Results

The pathological findings of surgical specimens from radical neck dissection were consistent with the typical histologic characteristics of branchiogenic carcinoma. Normal squamous epithelium and dysplastic and cancerous portions showed strong p16^{INK4a} immunoreactivity. Expression of p16^{INK4a} was also observed in all 9 nodal metastases in neck dissection specimens. The presence of HPV-16 in the tumor was confirmed by both PCR and in situ hybridization. The viral load of HPV-16 was $3.01 \times 10^7/50$ ng genomic DNA, and the E2/E6 ratio was 0.13. ISH study demonstrated that the signals located in nuclei of basal to granular layers of the cyst wall. These findings suggested that the integration state was judged to be the mixed type.

Conclusion

This is the first report of branchiogenic carcinoma associated with high-risk type human papillomavirus infection.

HN 08-08

: Diagnosis of HPV driven head and neck cancer: Comparing p16 based algorithms with the RNAscope HPV-test

H. Mirghani¹, **O. Casiraghi**², **M. Xiao-Jun**³, **J. Lacau St Guly**⁴, **C. Badoual**⁵, **I. Borget**⁶, **V. Dalstein**⁷, **P. Vielh**⁸

¹Department of Otolaryngology – Head and Neck Surgery, Gustave Roussy Cancer Campus, 114 rue Edouard Vaillant, Villejuif, France (France),

²Department of Pathology, Gustave Roussy Cancer Campus, 114 rue Edouard Vaillant, Villejuif, France (France), ³Advanced Cell Diagnostics, 3960 Point Eden Way, Hayward, CA 94545, USA (United States of America), ⁴Department of Otolaryngology-Head and Neck Surgery, Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, France. (France), ⁵Department of Pathology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, France. (France), ⁶Department of Biostatistics and Epidemiology, Gustave Roussy Cancer Campus and University Paris-Sud, Villejuif, France (France), ⁷INSERM UMR-S 903, , SFR CAP-Santé FED 4231, Université de Reims Champagne-Ardenne, F-51100 Reims, France (France), ⁸Department of Biopathology, Gustave Roussy Cancer Campus, 114 rue Edouard Vaillant, Villejuif, France (France)

Background / Objectives

Accurate identification of HPV-driven oropharyngeal cancer (OPC) is a major issue and none of the current diagnostic approaches is ideal. An in situ hybridization (ISH) assay that detects high-risk HPV E6/E7 mRNA, called the RNAscope HPV-test, has been recently developed. Studies have suggested that this assay may become a standard to define HPV-status.

Methods

To further assess this test, we compared its performance against the strategies that are used in routine clinical practice: p16 immunohistochemistry (IHC) as a single test and algorithms combining p16-IHC with HPV-DNA identification by PCR (algorithm-1) or ISH (algorithm-2).

Results

105 OPC specimens were analyzed. The prevalence of HPV-positive samples varied considerably: 67% for p16-IHC, 54% for algorithm-1, 61% for algorithm-2 and 59% for the RNAscope HPV-test.

Discrepancies between the RNAscope HPV-test and p16-IHC, algorithm-1 and 2 were noted in respectively 13.3%, 13.1%, and 8.6%.

The 4 diagnostic strategies were able to identify 2 groups with different prognosis according to HPV-status, as expected. However, the greater survival differential was observed with the RNAscope HPV-test [HR: 0.19, 95% confidence interval (CI), 0.07–0.51, $p=0.001$] closely followed by algorithm-1 (HR:0.23, 95% CI, 0.08–0.66, $p=0.006$) and algorithm-2 (HR:0.26, 95% CI, 0.1–0.65, $p=0.004$). In contrast, a weaker association was found when p16-IHC was used as a single test (HR: 0.33, 95% CI, 0.13–0.81, $p=0.02$).

Conclusion

Our findings suggest that the RNAscope HPV-test and p16-based algorithms perform better than p16 alone to identify OPC that are truly driven by HPV-infection. The RNAscope HPV-test has the advantage of being a single test.

HN 09-01

Human Papillomavirus Genotype and Oropharynx Cancer Survival

M. Goodman

Cedars Sinai Medical Center (United States of America)

Background / Objectives

The presence of human papillomavirus (HPV) DNA in oropharyngeal squamous cell cancer (OPSCC) tissue appears to be a strong predictor of improved prognosis, but this observation has not been explored in a population-based sample with generalisable findings.

Methods

Follow-up data from a large sample of OPSCC patients identified through six population-based cancer registries in the United States of America (USA) were used to characterise the association of tumour HPV status with survival.

Results

HPV DNA was detected in tumour tissue from 71% (378 in 529) of the OPSCC patients. A total of 65% of patients with HPV16-associated tumours survived 5 years compared to 46% of patients with other HPV types and 28% of patients with HPV-negative tumours (p log-rank test <0.0001). The OPSCC patients with detectable HPV16 DNA had a 62% reduced hazard of death at 5 years, and patients with other HPV types had a 42% reduced hazard of death at 5 years compared to HPV-negative patients. Compared to non-Hispanic Whites, Blacks with OPSCC had a 2.6-fold greater risk of death at 5 years after adjustment for HPV status and other prognostic variables. Both surgery and radiation therapy were associated with a reduced 5-year risk of death, but no evidence was found for an interaction between HPV status and radiotherapy or surgery on survival time.

Conclusion

Data from this US study suggest that HPV16-positive OPSCC patients survive longer than HPV-negative patients regardless of treatment, highlighting the prognostic importance of HPV status for this malignancy. Optimal treatment regimens for OPSCC could be tailored to each patient's HPV status and prognostic profile.

HN 09-02

Oral Cancer Screening on Oral Rinse Samples using Quantitative E6, E7 mRNA and Flow Cytometry

A. Chargin¹, R. Morgan¹, D. Gupta², B. Patterson¹

¹IncellDx, Inc. (United States of America), ²CureHealth (India)

Background / Objectives

HPV (human papilloma virus) infects epithelial cells causing lesions with the potential to progress into carcinoma. This is most commonly seen in the area of the cervix where HPV infection leads to deregulation of the cell cycle through the expression of E6/E7 oncogenes. The same mechanism is functional in head and neck cancer. To that end, we report the application of HPV OncoTect (IncellDx Inc, Menlo Park, CA) to determine the expression of E6, E7 mRNA in oral cancer.

Methods

We screened for E6, E7 mRNA overexpression by flow cytometry on oral rinse samples from 85 patients including 5 with oral cancer. Samples were collected by rinsing the mouth with 1-2 mL DPBS. Cells were pelleted and then fixed in 1 mL IncellFP followed by hybridization to a E6, E7 mRNA probe cocktail and then collected on a BD Accuri C6 cytometer.

Results

In 85 individuals undergoing oral screening for various risks of oral cancer, 32/85 or 38% were positive for E6, E7 mRNA. In comparison, 3/5 (60%) of individuals with oral cancer were positive for E6, E7 mRNA.

Conclusion

Quantitative intracellular E6, E7 mRNA determined by flow cytometry (HPV OncoTect) is a viable method to screen for HPV related oral cancer. It remains to be determined what the prognostic value of E6, E7 mRNA expression is in this setting.

HN 09-03

HPV DETECTION IN HEAD AND NECK CARCINOMAS: EVALUATION OF IN SITU HYBRIDIZATION, P16 IMMUNOHISTOCHEMISTRY AND GENEXPERT HPV ASSAY.

R. Cerutti, D. Furlan, C. Facco, A.M. Chiaravalli, F. Sessa, C. Riva

**Anatomic Pathology, Department of Surgical and Morphological Sciences,
University of Insubria and ASST-Sette Laghi, Varese (Italy)**

Background / Objectives

The identification of Human Papilloma Virus (HPV) is very useful to identify a subset of head and neck cancer, especially oropharyngeal, with peculiar biological characteristics and favorable prognosis. In this setting it is important to have a sensitive and reliable method to identify high-risk (HR)-HPV types in formalin fixed-paraffin embedded (FFPE) specimens. We sought to define the optimal technical approach to identify HPV-related head and neck carcinomas, in order to reach adequate sensitivity and specificity with a method applicable to diagnostic laboratory routine. In addition, we wanted to evaluate if HR-HPV could be involved in non-oropharyngeal head and neck cancers.

Methods

We analyzed a total of 101 FFPE oropharyngeal squamous cell carcinomas (SCC), diagnosed from 1998 to 2015 in our institution and for comparison 43 non-oropharyngeal SCC (31 of the oral cavity, 6 of the larynx and 6 from other head and neck sites). All the samples were tested with HR-HPV In Situ Hybridization (ISH) Kit (Roche) and p16 Immunohistochemistry (IHC) (CINtec Histology v-kit Roche). On a subset of 34 oropharyngeal SCC we also performed the cartridge-based GeneXpert HPV assay (Cepheid), a qualitative real time PCR assay validated to detect HR-HPV DNA in cervical cytological specimens.

Results

With ISH analysis, we identified HR-HPV positive cases in 32/101 (32%) oropharyngeal SCC and in 0/31 SCC of the oral cavity. In addition, we identified two HR-HPV positive SCC, one in the larynx and one in the nasal cavity. We obtained a good correlation between p16 and ISH, given that 118 of 133 comparable cases (89%) gave concordant results. The discordant cases included 12 out of 133 cases (9%) that were p16+/ISH- and 3 cases that were p16-/ISH+ (2%). In the cases studied with PCR, we obtained valid results in 28/34 cases (82%), with a 86% concordance with ISH analysis (24/28

cases). Three of 4 discordant cases were ISH-/PCR+ with a strong p16 immunoreactivity, evidencing a slightly better sensitivity of PCR in HPV detection. The latter discordant case was ISH+/PCR-, and showed no p16 immunoreactivity. Interestingly, the viral type identified was type 16 in 17/19 PCR positive cases.

Conclusion

The optimal strategy for the accurate identification of HR-HPV positive head and neck carcinomas is to combine the sensitivity of p16 IHC analysis and the specificity of ISH or PCR analysis. PCR showed a slightly greater sensitivity for HPV identification compared to ISH analysis: notably, GeneXpert HPV assay showed a good efficiency also starting from FFPE material. The impact of HPV in non-oropharyngeal head and neck SCC seems to be very limited in our series.

HN 09-04

STUDY OF HPV AND PRECANCEROUS LESIONS IN THE TONSILS (“SPLIT”): PRELIMINARY RESULTS

J.D. Combes¹, **V. Dalstein**², **G. Clifford**¹, **T. Gheit**¹, **M. Tommasino**¹, **C. Clavel**², **J. Lacau St Guily**³, **S. Franceschi**¹

¹International Agency for Research on Cancer, Lyon (France), ²INSERM UMR-S 903 / Université de Reims Champagne-Ardenne / CHU Reims, Laboratoire Pol-Bouin, Reims (France), ³Service d'ORL et chirurgie cervico-faciale, Hôpital Tenon AP-HP, Paris (France)

Background / Objectives

The SPLIT study aims to understand the natural history of HPV infection in the tonsils.

Methods

Since 2012, tonsils from children and adults non-cancer patients are being collected in 20 centres in France. For each patient, half of the resected tonsils are extensively brushed to collect exfoliated cells for detection of HPV and other potential oncogenic viruses and cytologic examination. A subset of 11 centers are also collecting rinse/gargling samples before tonsillectomy.

Results

To date close to 700 targeted patients have been included. Preliminary findings from the first batches of samples include the following: HR-HPV DNA was detected in 9 out of 500 tonsil brushing (of which four HPV16) and in 28 out of 209 gargles (of which 19 HR-HPV including 10 HPV16). Among 154 patients with both gargle and brushing specimens tested, out of 23 patients HPV-positive in gargle, only three were also positive for the same HPV type in tonsil brushing.

Conclusion

Preliminary results suggest that HPV is rarely detected in tonsil tissue, although more frequently detected in gargles. HPV testing of oral cells through gargling may be therefore an interesting epidemiological tool but it is little representative of the presence of HPV infection in the head and neck site most prone to HPV carcinogenesis. Final results of HPV infection and cytology will be presented.

HN 09-05

METHYLATION LEVELS IN HPV16 E2 BINDING SITES 3 AND 4 ARE RELATED TO HISTOLOGICAL SUBTYPE AND SURVIVAL IN A COHORT OF OPSCC PATIENTS

M.S. Kalteis¹, M. Reuschenbach¹, S. Vinokurova², U. Keilholz³, A. Zakarneh⁴, I. Tinhofer-Keilholz⁵, M. Von Knebel Doeberitz¹, E.S. Prigge¹

¹Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg, Heidelberg (Germany), ²Institute of Carcinogenesis, NN Blokhin Cancer Research Center, Moscow (Russian federation),

³Comprehensive Cancer Center, Charité Universitätsmedizin, Berlin (Germany),

⁴Department of Otorhinolaryngology, Sankt Gertrauden Krankenhaus, Berlin (Germany), ⁵Clinic for Radiooncology, Charité Universitätsmedizin, Berlin (Germany)

Background / Objectives

The HPV16 upstream regulatory region (URR) undergoes shifts in methylation during cervical carcinogenesis. Four binding sites for E2 (E2BS), a key regulatory protein in HPV infection, are located in this region. Methylation of E2BS 3 and 4 is thought to promote uncontrolled expression of the HPV oncogenes E6 and E7 which drive carcinogenesis.

It has been shown previously that methylation at HPV E2BS3/4 allows for classification of subpopulations of oropharyngeal squamous cell cancer (OPSCC). In order to gain additional insight into the epigenetic regulation of HPV oncogene expression, we analyzed a series of tonsillar and base of the tongue carcinomas along with matched lymph node metastases and correlated E2BS3 and 4 methylation levels with clinical data.

We hypothesized that there might be distinct shifts in the binding sites' methylation during formation of metastases, possibly due to the selection and subsequent metastatic expansion of cell populations. Additionally, there might be subgroups of OPSCC with discernible differences in E2BS3 and 4 methylation and associated patient survival.

Methods

FFPE tissue from 67 HPV16 DNA+ and p16INK4a+ neoplastic lesions consisting of 42 OPSCC primaries with 25 matched lymph node metastases was obtained from St. Gertrauden-Krankenhaus, Berlin, Germany. DNA was bisulfite-converted and analyzed for methylation in five CpG sites in E2BS3/4 (positions 31-58) of the HPV16 URR by pyrosequencing. Differences in methylation levels among groups defined by clinical characteristics were assessed using ANOVA and Tukey's post-hoc tests. A

cut-off level for assorting samples into groups of low versus high methylation was established using hierarchical cluster analysis. Kaplan-Meier-curves and log-rank-tests were used to examine different overall (OS) and progression-free survival (PFS) among these groups.

Results

There was a trend towards lower methylation levels in E2BS CpGs in lymph node metastases compared to OPSCC primaries, which reached significance for CpG 43 in E2BS3 ($p=.022$).

Tumor histology was significantly associated with methylation: OPSCC with basaloid histology were significantly higher methylated than non-basaloid OPSCC considering confounding clinical factors.

High methylation levels (> 52% as established by cluster analysis) were associated with reduced OS and PFS.

Conclusion

OPSCC histological subtypes apparently harbor differences in HPV16 E2BS3/4 methylation levels which could be responsible for changes in the specific regulation of HPV E6/E7 oncogene expression, thereby potentially modifying the course of the disease.

Differences in URR methylation could be a marker for predicting survival in specific OPSCC subpopulations.

HN 09-06

ASSOCIATION OF HPV INFECTION, XENOBIOTIC GENE POLYMORPHISM, MITOCHONDRIAL MUTATIONS AND TOBACCO WITH ORAL CANCER-A STUDY FROM NORTHEAST INDIA

R. Mondal

INSTITUTE OF ADVANCED STUDY IN SCIENCE & TECHNOLOGY (India)

Background / Objectives

Head and neck cancer (HNC) is one of the most common malignancies worldwide. Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and malignancy among HNC globally. Northeast India has one of the world's highest incidences of oral cancer; it is the most common malignancy among head and neck cancers (HNC), accounting for approximately 30%–40%. Tobacco consumption, alcohol use, smokeless tobacco products human papillomavirus (HPV) infection and glutathione S-transferase (GST) gene polymorphisms are the major risk factors for oral cancer, with smoking and alcohol having synergistic effects. HPV infection has been a prime suspect in the aetiology of OSCC due to its morphological association with SCCs and its ability to immortalize oral keratinocytes and bring about transformation of epithelial cells. Further, mitochondrial DNA (mtDNA) alterations are associated with various cancers, suggesting that it may be a critical contributing factor in carcinogenesis. Here, we investigated the association of tobacco–betel quid chewing, HPV infection, GSTM1-GSTT1 genotypes and mitochondrial D-loop mutations with OSCC.

Methods

The mutations from matched tissue samples of OSCC patients with control subjects were used for PCR and direct sequencing. PCR-based detection was done for high-risk HPV using a consensus primer Gp5+/Gp6+ and My09/My11 for amplifying HPV L1 gene fragments, and multiplex PCR was done for detection of GSTM1-GSTT1 polymorphism using CYP1A1 as an internal control. DNA from cervical cancer cell lines infected with known HPV types was used as positive controls. PCR products were analyzed by electrophoresis on 2.5% agarose gels stained with ethidium bromide. All possible precautions like sample processing and PCR reaction preparation in separate biosafety cabinet, sterile gloves changing after each batch of reactions, inclusion of negative controls in all PCR reactions, etc. were maintained to minimize contamination.

Conclusion

Our results suggest that the association of tobacco–betel quid chewing, null GST genotypes, HPV infection and mutations can be used as a possible biomarker for early detection and prevention of oral cancer. Furthermore, biochemical and molecular studies are necessary to determine the pathological significance of these associated somatic mutations. The most reliable way to prevent infection with either high-risk or low-risk HPV is by avoiding any skin-to-skin oral, anal or genital contact with another person. Those who are sexually active, long term, mutually monogamous relationship with an uninfected partner are the strategy most likely to prevent HPV infection. PCR must be employed in combination with histological detection for rapid, sensitive and specific detection of HPV, thereby facilitating early therapeutic decisions in suspected and histopathological negative cases, thus providing the clinicians the guidance for choosing an accurate treatment of HPV-infected advanced OSCC cases.

HN 09-07

DETECTION OF HPV 16 AND 18 ONCOPROTEINS WITH AN ONCOE6™ ORAL TEST IN FINE NEEDLE ASPIRATES OF CERVICAL LYMPH NODES FROM PATIENTS WITH HEAD AND NECK CANCERS

M. Chernesky¹, **J. Gilchrist**¹, **D. Jang**¹, **M. Arias**¹, **L. Doerwald-Munoz**², **M. Gupta**², **E. Young**², **S. Archibald**², **B. Jackson**², **A. Lytwyn**², **M. Smieja**², **J. Schweizer**³

¹St. Joseph's Healthcare/McMaster University (Canada), ²Juravinski Cancer Centre (Canada), ³Arbor Vita Corporation (United States of America)

Background / Objectives

Some head and neck squamous cell carcinomas (HNSCC) are causally associated with high-risk human papillomavirus (HR-HPV) genotypes 16 and 18. Current HPV detection methods include surrogate p16 immunohistochemical (IHC p16) staining, in situ hybridization, and nucleic acid amplification assays. Arbor Vita Corp has developed an OncoE6™ Oral Test to detect the presence of HPV 16 and 18 E6 oncoproteins in samples. The study objective was to determine if the OncoE6™ Oral Test could detect HPV 16/18 E6 oncoproteins in cervical lymph node fine needle aspirates (FNAs), base of tongue (BOT) swabs, and saliva samples from patients with HNSCC who had HR-HPV E6/E7 mRNA in FNAs and/or IHC p16 positive tumors.

Methods

Clinic patients (n=68; 61 males and mean age 59.8 years), with confirmed or suspected HNSCC consented to collection of FNA, BOT swab, and saliva samples. FNAs were collected and preserved in 4 ml of PreservCyt ThinPrep fluid (TP; Hologic). A BOT swab was collected into a 15 ml tube. Saliva (1 ml) was collected into an OMNIgene Discover saliva collection kit (OM-505; DNA Genotek) for mRNA testing and another 1 ml was collected into a 15 ml tube for OncoE6™ testing. In the laboratory, TP was added to the FNA (1 ml) and BOT swab (5 ml), additional OMNIgene preservative (4 ml) was added to the Genotek saliva sample. The Aptima HPV (AHPV) assay was performed on 1 ml of sample added into 2.9 ml Aptima specimen transport media. For the OncoE6™ test, 1 ml of each sample type was used; total test time was 2.5 h.

Results

Primary tumours were p16 positive in 58 of 68 patients. Overall agreement of the OncoE6™ Oral Test on FNA with p16 staining of tumors was 73.5% (50/68, k=0.40). IHC p16 staining of tumors and AHPV testing of FNA had an overall agreement of 83.8% (57/68, k=0.56). The AHPV assay detected E6/E7 HPV mRNA in 47 FNA samples, of which 39 were positive by OncoE6™. OncoE6™ and AHPV testing of FNAs showed 86.8% (59/68, k=0.72) overall agreement. The sensitivity of the OncoE6™ test for detecting HPV infected patients who were both p16 and AHPV positive was 83.0% (39/47); the specificity was 95.2% (20/21). AHPV mRNA was positive in 24 saliva samples, 3 of which were positive by OncoE6™. Thirteen BOT swabs were positive by AHPV, while none were positive by OncoE6™.

Conclusion

The OncoE6™ Oral Test was easy to perform and detected 83.0% of patients who had both p16 IHC positive tumors and AHPV E6/E7 mRNA positive FNA. The OncoE6™ Oral Test is the first commercial test for the detection of E6 oncoproteins in FNA and its strong agreement of 86.8% (k=0.72) with our reference standard should make it useful as a diagnostic test for HPV induced HNSCC.

HN 09-08

IMMUNE INFILTRATION OF ORAL PHARYNGEAL SQUAMOUS CELL CARCINOMAS (OPSCC) AND PROGRAMMED CELL DEATH LIGAND-1 (PD-L1) EXPRESSION: RELATIONSHIP TO CLINICAL OUTCOME

P. Stern ¹, K. Oguejiofor ², H. Galletta ², S. Dovedi ³, C. West ²

¹Women's Cancer, Institute of Cancer Sciences, University of Manchester (United kingdom), ²Translational Radiobiology Group, Institute of Cancer Sciences, University of Manchester (United kingdom), ³Targeted Therapy Group, Institute of Cancer Sciences, University of Manchester (United kingdom)

Background / Objectives

Tumours infiltration by CD8 T cells is linked to favourable outcomes in several cancers. However, chronic activation of T-cells leads to up-regulation of PD-1 that on interaction with its ligand PD-L1 can modulate effector function. Importantly, blocking antibodies to PD-1 or its ligand have shown efficacy in treatment of some cancers. Expression of PD-L1 in the tumour microenvironment could therefore be a biomarker of potential response to checkpoint inhibitors.

Methods

Here, the densities of T cells, macrophages and their PD-1 and PD-L1 expression are assessed within different tumour microenvironments by multiplex immune-fluorescent labelling in 124 OPSCC and association with HPV status and clinical outcomes evaluated.

Results

In HPV+ OPSCC patients CD8 density in the stroma correlates with significantly improved survival. By contrast, HPV- OPSCCs have a lower CD8 infiltration and a higher proportion of PD-1+ CD8 T cells and PD-L1+CD68 macrophages in their stroma. These data are consistent with checkpoint modulation leading to poorer clinical outcome in HPV- patients. However within this group, the infiltration of CD68 especially PD-L1+CD68 cells and increased expression of PD-L1 in the stroma are all associated with significantly improved survival. This implies the best control of HPV- tumours is driven by macrophage activity.

Conclusion

CD8 T cell and CD68 PD-L1+ macrophage densities differ between HPV+ and negative OPSCC and are associated with differing outcomes. This may reflect a different natural history with altered immune control mechanisms apparent in the cancers at time of diagnosis. No simple pattern of PD-L1 expression is utilizable as a biomarker for either prognosis or treatment selection in OPSCC.

CS 01-01

“HPV TEST PERFORMANCE IN VACCINATED WOMEN”

R. Bhatia ¹, K. Kavanagh ², H. Cubie ¹, I. Serrano ¹, H. Wennington ¹, M. Hopkins ³, J. Pan ², K. Pollock ², T. Palmer ⁴, K. Cuschieri ⁵

¹University of Edinburgh (United kingdom), ²University of Strathclyde (United kingdom), ³Royal Liverpool University Hospital (United kingdom), ⁴Univeristy of Edinburgh (United kingdom), ⁵Royal Infirmary of Edinbugh (United kingdom)

Background / Objectives

The management of cervical disease is changing worldwide as a result of HPV vaccination and the increasing use of HPV testing for cervical screening. However, the impact of vaccination on the performance of HPV based screening strategies is largely unknown. Projects such as the SHEVa (Scottish HPV Prevalence in Vaccinated women) studies are designed to gain insight into the impact of vaccination on the performance of clinically validated HPV assays.

Methods

Samples collated from women attending for first cervical smear who had been vaccinated as part of a national “catch up” programme were tested with three clinically validated HPV assays (2 DNA and 1 RNA) and one highly sensitive, epidemiologically orientated assay. Overall HR-HPV and type specific positivity was assessed in the total population and according to underlying cytology and compared to a demographically equivalent group of unvaccinated women. A separate analysis of test performance in samples obtained from immunised women and enriched for cytological abnormalities was also performed

Results

HPV prevalence was significantly lower in vaccinated women and was influenced by assay-type, reducing by 23-25% for the DNA based assays and 32% for the RNA assay ($p=0.0008$). All assays showed over 75% reduction of HPV16 and/or 18 ($p<0.0001$) whereas the prevalence of non 16/18 HR-HPV was not significantly different in vaccinated vs unvaccinated women. In women with low grade abnormalities, the proportion associated with non 16/18 HR-HPV was significantly higher in vaccinated women ($p<0.0001$). Data on clinical performance of the assays using the disease-enriched samples will be presented

Conclusion

Clinically validated HPV assays are affected differentially when applied to vaccinated women, dependent on assay chemistry. The increased proportion of non HPV16/18 infections may have implications for clinical performance, consequently, longitudinal studies linking HPV status to disease outcomes in vaccinated women will provide welcome insight.

CS 01-02

Impact of HPV immunisation on infection and disease in a screened population

K. Pollock¹, K. Cuschieri²

¹Health Protection Scotland (United kingdom), ²Scottish HPV Reference Laboratory (United kingdom)

Background / Objectives

A national HPV immunisation programme was initiated in Scotland in 2008 for 12-13 year olds with a 3 year “catch up” for those under the age of 18. In tandem with the national immunisation programme, a programme of longitudinal HPV surveillance was also initiated. Key elements of surveillance were yearly sampling and HPV genotyping of women attending for their first smear and the monitoring of both low and high grade lesion prevalence through interrogation of national databases. As age at screening debut is currently 20 in Scotland, we are able to robustly determine the impact of a national immunisation programme on rates of HPV infection and HPV associated disease.

Methods

Liquid-based cytology (LBC) samples from women attending their first cervical smear were genotyped for HPV and data linkage enabled HPV prevalence to be stratified by immunisation status. In addition, we analysed data from the National Colposcopy Clinical Information and Audit System (NCCIAS), a national colposcopy database which contains data on referral cytology, interventions and histology results associated with any colposcopy visit.

Results

By linking vaccination, cervical screening, and HPV testing data, over the study period we found a decline in HPV types 16 and 18, significant decreases in HPV types 31, 33, and 45 (suggesting cross-protection), and a non-significant increase in HPV 51. In addition, among non-vaccinated women, HPV types 16 and 18 were significantly lower in 2013 than in 2009. We also observed significant reductions in all grades of cervical intraepithelial neoplasia associated (55% reduction of CIN 3, 50% reduction of CIN 2 and a 29% reduction of CIN 1) with 3 doses of the HPV vaccine.

Conclusion

This is one of the first studies to show demonstrable impact of the bivalent vaccine on HPV genoprevalence and associated disease at the population level. These data are very encouraging for countries that have national programmes which have achieved high vaccine uptake, as it suggests that herd protection in the unimmunised may also be realisable.

References

Cameron RL, Kavanagh K, Pan J et al. (2016) Human papillomavirus prevalence and herd immunity after introduction of vaccination program, Scotland, 2009–2013.

Emerg Infect Dis 22: 56–64.

Pollock KG, Kavanagh K, Potts A, Love J, Cuschieri K, Cubie H, Robertson C, Cruickshank M, Palmer TJ, Nicoll S, Donaghy M (2014) Reduction of low- and high-grade cervical abnormalities associated with high uptake of the HPV bivalent vaccine in Scotland.

Br J Cancer 111: 1824–1830.

CS 01-03

Modelling the impact of vaccination on alternative screening policies in Scotland

K. Kavanagh¹, A. Neilson², M. Cruickshank³, S. Cotton³, C. Robertson³

¹Department of Mathematics and Statistics, University of Strathclyde, Glasgow, G1 1XH (United kingdom), ²Health Economics Research Unit, University of Aberdeen, Aberdeen, AB25 2ZD (United kingdom), ³Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen AB25 2ZD (United kingdom)

Background / Objectives

With an established UK school based HPV immunisation programme, the approach to cervical screening requires modification to optimise cancer prevention, but retain clinical and economic efficiency.

Methods

A Markov simulation model has been constructed for a cohort of 35,000 women who are followed from age 12 until age 75. The model considers possible acquisition of HPV and regression or progression of disease in a similar fashion to the natural history model of Choi, 2009. Women are assigned a risk level for sexual activity and a deprivation quintile. Current national data indicates these factors impact on risk of HPV infection, uptake of vaccination and attendance at screening.

The model estimates the rates of cervical cancer, rates of CIN, numbers of colposcopies per year and the relative costs of different screening strategies and subsequent treatments in the vaccinated cohort of women.

Results

We will demonstrate results for alternate screening strategies for referral to colposcopy – primary HPV screening either alone, or triaged by cytology. These are compared to the current practice of cytology only with HPV test of cure. We consider variation of the age and frequency of screening and examine the impact on the burden of cervical disease.

Conclusion

Whilst there are uncertainties present in the modelling process, the results of these models allow us to consider various “what if” scenarios and help to instruct policy on the future of the cervical screening programme in Scotland.

References

Choi et al, (2009) Transmission dynamic modelling of the impact of human papillomavirus vaccination in the United Kingdom. *Vaccine* 28: 4091-4102

CS 01-04

What does the vaccine era change for the clinician?

M. Cruickshank¹, S. Cotton¹, K. Cuschieri², K. Pollock³, K. Kavanagh⁴, C. Roberston⁴

¹Univeristy of Aberdeen (United kingdom), ²NHS Lothian (United kingdom), ³Health Protection Scotland (United kingdom), ⁴University of Strathclyde (United kingdom)

Background / Objectives

Worldwide, an increasing number of countries have implemented HPV vaccination programmes or the vaccine is available. Those with cervical screening, continue to provide secondary prevention to gain most health benefit. The early effect of the HPV vaccination on cervical abnormalities from Australia (1) has shown a significant decrease in high-grade cervical abnormalities in younger women. More recent results from the UK on the prevalence of HPV16/18 and CIN3 from surveillance by Health Protection Scotland demonstrate the prevalence of HPV16 and 18 has decreased in women aged 20 from 29.8% to 13.6%. A reduction was also seen in other hrHPV types, HPV 31, 33 and 45, suggesting cross protection of closely related genotypes. A significant reduction in diagnoses of CIN 1 (RR[1] 0.71), CIN 2 (RR 0.5) and CIN 3 (RR 0.45) was observed in women who received three doses of vaccine compared with unvaccinated women.

Such changes in HPV prevalence and HPV related disease in the screening target population, will impact on the screening performance and disease detection and prevention and should inform service providers.

Methods

A programme of research incorporating observational studies from linkage of routinely collected clinical data and additional HPV DNA and genotyping studies of women attending for cervical screening in Scotland was used to demonstrate the impact of HPV vaccination in the catch-up programme of women aged 14-18 years once they attend for routine cervical screening from age 20 years.

Conclusion

Observational and interventional data from Scotland shows a reduction in the absolute numbers and relative proportions of young women referred to colposcopy from the cohort of women offered HPV immunisation. This has reduced colposcopy referrals and activity with less procedures and treatments performed. The lower incidence of abnormal cytology and CIN is already impacting on

the current screening strategies and performance of colposcopy. In countries with vaccination and screening programmes, screening programmes need to review their criteria for identification of an appropriately high risk group who would benefit from colposcopy and avoid potential harm from screening and interventions in low risk women.

CS 02-03

Colposcopy Trainers; training trainers and assessing competency

M. Cruickshank

Univeristy of Aberdeen (United kingdom)

Background / Objectives

Colposcopy remains central to disease ascertainment and treatment for women identified through primary screening with or without triages high risk. Quality assurance (QA) of colposcopy services is fundamental to the quality and performance of any cervical screening programme. QA requires not only monitoring of the service provision but training of colposcopists and colposcopy trainers themselves. This requires a recognised training programme with transparency of the assessment of competency on completion of training and accountability for achieving competency for independent clinical practice as well as a process to ensure that competency continues beyond the time of training.

Methods

QA of training and assessment can be managed through evaluation of current medical education literature, peer review and support from specialist societies, colleges or regulatory bodies. Ideally any programme should perform their own internal and external review and validation processes.

Conclusion

The focus will be on the requirements of the trainer and the colposcopy service to provide quality training to allow the trainee to achieve skills and attributes of an independant practitioner. The assessment of competency will look at both educational and practical issues and consider different methods of assessing competency for independent practice.

CS 02-04

Developing the role of lead in colposcopy

J. Tidy

Sheffield Teaching Hospitals NHS Foundation Trust (United kingdom)

Background / Objectives

The three components of any cervical screening programme - cytology, histopathology and colposcopy are prone to subjectivity and hence variation in practice. To optimise care and outcomes it is essential that these variations are minimised for the benefit of women who enter a screening programme. Quality assurance (QA) strategies can help to achieve this aim. The role of a lead in colposcopy is pivotal to the success of a QA programme.

Methods

The role requires the development of standard operating procedures (SOPs), local guidelines and introduction of a local multidisciplinary meeting to discuss challenging and difficult cases. Ideally the role of lead in colposcopy should be associated with administrative time to fulfill the job. Local guidelines and SOPs will in the greater part reflect national standards.

Results

The latest guidance was published in March 2016 and deals with both the organization of the screening programme – age range, screening intervals, primary cytology with reflex HPV testing for low grade cytology, colposcopic diagnosis, conservative management, treatment, glandular neoplasia, immunosuppression, follow up and test of cure post treatment. The performance of an individual colposcopist can be difficult to quantify. Currently it is not possible to assess colposcopic opinion and many studies have shown wide inter and intra observer error when colposcopic images are evaluated. Therefore we use a range of surrogate parameters to assess individual colposcopic performance including: positive predictive value for a high grade colposcopic impression, excision of TZ in one piece, depth of excision, complications rates, rates for treatment under GA, histological failure rates 12 months after treatment.

Conclusion

To ensure that a screening programme is effective and delivers an equitable service to all women QA systems can play an effective role. QA across a clinic can assess overall performance for comparison with other clinics, across a region of a country and nationally. In the UK there are regional and national QA structures to deliver and monitor the process. In a national programme this information can be used to improve standards of care.

References

1. Cervical screening: programme and colposcopy management NHSCSP No.20 3rd ed. www.gov.uk/government/publications/cervical-screening-programme-and-colposcopy-management

CS 02-05

Quality Control in Colposcopy: Canadian Experience

M. Roy

Laval University (Canada)

Quality control is mandatory in a cervical cancer screening programme, including colposcopy. Nowadays, we often hear that blind four-quadrant biopsies, after an abnormal Pap test, give better results than colposcopic evaluation. Lack of quality control can explain the poor 50% of correlation between colposcopy and blind multiple biopsies. In Canada, quality control in colposcopy was initiated as early as 1973 and over the years, colposcopic impression mirrored the referral cytology diagnosis in 90% of the cases. Quality control should cover the following: training of colposcopists, indications for referral, number of new consultations annually, types of treatments, correlation between colposcopy impression and histopathology results. When a colposcopist does not meet the standard, training sessions should be mandatory. Since HPV vaccination programmes are in place, there will be fewer references for high grade lesions. Quality control will be more important.

CS 03-01

Principle of risk-based colposcopy

N. Wentzensen

Division of Cancer Epidemiology and Genetics National Cancer Institute - Bethesda (United States of America)

Background / Objectives

Colposcopy is the centerpiece of most cervical cancer screening programs. In screen-positive women, colposcopic impression and biopsy results determine further management or treatment. Colposcopy-biopsy practice ranges from taking single biopsies from the worst appearing lesion to routinely taking multiple random biopsies independent of colposcopy impression.

There is a wide range of risk of cervical precancer among women who are referred to colposcopy, suggesting that colposcopy-biopsy procedures could be adapted to the underlying risk. Several test results, including cytology, HPV genotype, and biomarkers like p16/Ki-67 cytology may be available at colposcopy. Together with colposcopy impression, they can provide strong risk stratification in a colposcopy population. We evaluated this approach in the Biopsy Study among 700 women referred to colposcopy at the University of Oklahoma.

In strata defined by HPV testing, cytology, and colposcopy impression, the absolute risk of precancer ranged from 2.4% for women with <HSIL cytology, no HPV16 and normal colposcopy impression to 75.9% for women with HSIL, HPV16 and high grade colposcopy impression. When restricting to cytology and colposcopy impression, the absolute risk ranged from 3.2% for women with <HSIL cytology and normal colposcopy impression to 71.2% for women with HSIL and high grade colposcopy impression. Similarly, when using HPV genotype and colpo impression, the absolute risk ranged from 5.1% for women without HPV16 and normal colposcopy impression to 76.1% for women with HPV16 and high grade colposcopy impression. We observed similar risk stratification for combinations of p16/Ki-67 cytology and colposcopy impression (range from 1.4%-68.8%).

Risk assessment at colposcopy can inform colposcopy-biopsy practice, including optimal number of biopsies, and guide management. In a low-risk group, a normal colposcopy confers higher reassurance that CIN3+ is not present. In the highest risk groups, immediate treatment without biopsy confirmation could be considered according to current guidelines. In the future, colposcopy populations will change when primary HPV screening and new triage strategies are implemented. Furthermore, with HPV-vaccinated women increasingly entering the screening age, women referred to colposcopy generally will have a lower risk of precancer. A risk-based approach will allow developing evidence-based guidelines for colposcopy that anticipates the changing populations.

CS 03-02

Risk based colposcopy - UK experience

M. Cruickshank

Univeristy of Aberdeen (United kingdom)

Background / Objectives

The NHS Cervical Screening Programme in England provides evidence based guidelines for colposcopy and programme mangement which are used through out the UK. Clinical services may have local protocols but most practice is based on the guidelines of document 20.

Methods

Guidelines are developed and edited by UK colposcopy experts based on literature search and review and grading of available evidence.

Conclusion

Quality assurance varies throughout the UK but each country has adopted a system which included key performance indicators based on the national guidelines to ensure quality of care for women which balances benefits and disbenefits of screening.

CS 03-03

THE DETECTION OF CIN2+ AFTER AN ABNORMAL PAP-SMEAR AND hrHPV POSITIVITY USING REPEAT CYTOLOGY, hrHPV GENOTYPING AND COLPOSCOPIC IMPRESSION

A. Leeman¹, A. Kasius¹, M. Del Pino², A. Rodriguez², B. Ter Harmsel³, M. Van De Sandt¹, A. Torne², J. Ordi⁴, F. Van Kemenade⁵, D. Jenkins¹, W. Quint¹

¹DDL Diagnostic Laboratory, Visseringlaan 25, 2288 ER Rijswijk (Netherlands),
²Institute of Gynecology, Obstetrics and Neonatology, Hospital Clínic Clínic— Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona (Spain), ³Roosevelt Clinic, Rooseveltstraat 67, 2321 BL, Leiden (Netherlands), ⁴Department of Pathology, Hospital Clínic, Barcelona (Spain), ⁵Erasmus Medical Center, Department of Pathology, PO Box 2040, 3000 CA Rotterdam (Netherlands)

Background / Objectives

In HPV screening, most women testing positive in cytology and hrHPV will be offered colposcopy. Colposcopy has a variable sensitivity, influenced by the expertise of the colposcopist and the number of biopsies taken. Information collected prior to or during colposcopy may increase effectiveness of CIN2+ detection i.e. decision making about treatment and follow-up.

Objective: To compose risk profiles of CIN2+ based on results of intake cytology, hrHPV genotyping and colposcopic impression for treatment decision support.

Methods

From a population of women referred to colposcopy after an abnormal PAP-smear, 682 hrHPV positive women were selected retrospectively. At the clinic, women had a physician-taken intake smear and a colposcopy with up to 4 biopsies. Physician-taken smears were tested with the clinically validated GP5+/6+-EIA-Luminex system and cytological analysis was performed. Colposcopy findings were described following the criteria of the IFCCP. Biopsies were reviewed by a local pathologist. Combinations of findings identifying a high risk of CIN2+ and a low risk of CIN2+ were examined.

Results

Among the 682 women, there were 94 (13.8%) women with an ASC-US referral smear, 240 with LSIL (35.2%) and 348 (51.0%) with \geq HSIL. In 359 (52.6%) women a CIN2+ lesion was detected in the biopsy. CIN2+ was detected in 34.0% of the women in the ASC-US group, in 30.4% in the LSIL group and in 73.0% in the \geq HSIL group.

Intake cytology, hrHPV genotype and colposcopic impression as individual factors all significantly increased the risk of CIN2+ after \geq HSIL cytology at referral (intake cytology \geq HSIL 85.0%, HPV16/18 82.4%, colposcopic impression \geq CIN2 84.5%). When combining the factors, a combination of \geq HSIL intake cytology and \geq CIN2 colposcopic impression resulted in significant increase of risk of CIN2+ among the women with \geq HSIL referral cytology (93.2%). For women with ASC-US and LSIL referral cytology with \geq HSIL intake cytology and \geq CIN2 colposcopic impression, the risk of CIN2+ was 71.4% and 92.6%, respectively. This combination also resulted in a significant decrease of risk of CIN2+ for women with LSIL referral cytology followed by \leq LSIL intake cytology and \leq CIN2 colposcopic impression (13.0%).

Conclusion

The combination that yielded the highest increase in risk was intake cytology combined with colposcopic impression. Women with \geq HSIL referral cytology, \geq HSIL intake cytology and \geq CIN2 colposcopic impression had a risk of CIN2+ of 93.2%. Women with persistent low-grade cytology and matching colposcopic impression were at a low, but not negligible risk. A combination of other risk factors, such as methylation status, might increase and/or decrease the risk of CIN2+.

CS 03-04

A meta-analysis of the accuracy of hrHPV testing and other markers to detect cervical precancer in women with ASC-H

L. Xu, M. Arbyn

Belgian Cancer Centre/Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels (Belgium)

Background / Objectives

Management of women with a cytological diagnosis of atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) includes immediate referral to colposcopy because of its high associated risk of underlying high-grade cervical intraepithelial neoplasia (CIN). However, triage may reduce the burden of diagnostic workup and avoid overtreatment.

Methods

A systematic review and meta-analysis were conducted to evaluate the accuracy of hrHPV testing with Hybrid Capture-2 (HC2) or other assays, HPV16/18 genotyping and testing for other molecular markers for the detection of CIN grade two or worse (CIN2+) or CIN grade three or worse (CIN3+) in the management of women with ASC-H. The relative accuracy of the other hrHPV assays or molecular markers using HC2 as comparator was also evaluated. An additional question assessed was whether triage is useful given the relatively high pretriage probability of underlying precancer.

Results

The pooled absolute sensitivity and specificity of hrHPV testing with HC2 to detect CIN2+ (derived from 19 studies) was 93% (95% CI: 89-95%) and 45% (95% CI: 41-50%), respectively. The p16^{INK4a} staining (only 3 studies) has similar sensitivity (ratio=0.99, 95% CI:0.87-1.12) but superior specificity (ratio= 1.69, 95% CI: 1.39-2.06) compared to HC2 for detecting CIN2+. The average pre-test risk was 34% for CIN2+ and 20% for CIN3+. A negative HC2 result decreased this risk to 8% and 5%, whereas a positive result upgraded the risk to 47% and 28%.

Conclusion

A cytological result of ASC-H is associated with a high risk of cervical precancer, which justifies immediate referral for colposcopy. Results in this meta-analysis support a certain utility of

hrHPV DNA testings and in particular of p16^{INK4a} cyto-immunochemistry. A positive triage result does not alter the decision to refer, but those testing negative could be recalled for a repeat test 6-12 months later in countries with a conservative follow-up policy. Nonetheless, in countries with a low decision threshold for colposcopy referral, triage of ASC-H would be considered as not useful to orient diagnostic workup.

CS 03-05

Surgical management of vestibulitis by vestibulectomy

J. Paavonen

Helsinki University Hospital (Finland)

Background / Objectives

Vulvar vestibulitis syndrome (VVS), or vestibulodynia, is a complex pain syndrome which is increasingly recognised, and has a major impact on the quality of life of many women.

Multiple treatment modalities have been used with relatively poor success.

Vestibulectomy can be used in refractory cases of severe VVS if conservative therapies have failed.

Methods

Modified posterior vestibulectomy by day surgery will be described.

The vestibulectomy case series consisted of 117 women with severe VVS and no response to conservative therapy.

Both short-term and long-term outcomes are reported among 92 of the 117 women.

Results

Of the vestibulectomy cases, 18 developed minor postoperative problems, mean duration of wound pain was 14 days, mean duration of sick leave was 13 days, and a mean of 6 weeks was needed for full recovery.

Long-term outcome after a mean of 4 year follow-up showed that 76% were sexually active, Beck depression score was 4 (mean), general health score (EQ-5D) was 8 (mean), and 24 women had had delivery postoperatively.

Preoperative VAS was 9.0 (mean) and postoperative VAS was 2.0 (mean).

Overall rate of satisfactory response was 90%, and 89% of the women would have choosed the operation again.

Conclusion

Day surgery by posterior vestibulectomy is strikingly effective in refractory cases of vulvar vestibulitis syndrome.

CS 04-01

The risk of HPV vertical transmission routes

H. Trottier

Université de Montréal / Sainte-Justine Hospital (Canada)

Background / Objectives

Evidence suggests that newborns are exposed perinatally to HPV. Vertical transmission of HPV was first reported by Hajek in 1956 in a case of juvenile laryngeal papillomatosis. Confirmation of the perinatal transmission in different mucosa (genital, oral, conjunctival) was subsequently supported by several studies. Previous studies have reported widely varying rates of infection in newborns, with estimates ranging from 4 to 79% among days old infants born to mothers testing positive for HPV during pregnancy. More recent studies reported that perinatal transmission (oral and/or genital and/or conjunctival) is between 4-22%.

Methods

Most cases of vertical transmission may occur at delivery through direct contact of the foetus and maternal infected cells during vaginal delivery or at caesarean section following early membrane rupture. Transmission in utero through semen or ascending infection from mother's genital tract is also possible. Peri-conceptual transmission could occur since HPV DNA has been detected in 8-64% of the seminal fluid and spermatozoa. Moreover, HPV DNA has been detected in the endometrium and ovaries. Transplacental transmission has also been supported as HPV DNA is detected in amniotic fluid with detection rates varying between 15-65%. HPV DNA has also been found in placental cells and in cord blood. The detection rate of HPV DNA in trophoblastic cells varies from 0 to 60% and between 0-13% in cord blood cells. Recently, HPV DNA has been detected in breast milk with a rate varying between 2-8%. This suggests that HPV could be transmitted vertically to neonates through breastfeeding. Finally, horizontal transmission just after delivery can occur via digital contacts.

Conclusion

This suggests that HPV could be transmitted vertically to neonates through several modes of transmission.

CS 04-02

THE CYTOLOGY IN PREGNANCY - THE IMPORTANCE OF PERFORMING PAP IN PREGNANCY AND PROBLEMS OF CORRECT DIAGNOSIS

K. Syrjänen

Chief Medical Director, Biohit HealthCare Oyj (Finland)

Background / Objectives

Pregnancy itself is not a contraindication to performing a Pap smear. It is recommended that Pap smears be offered to women where appropriate i) until at least 28 weeks of pregnancy, and ii) in special cases, during the third trimester (particularly, if she is likely to have difficulty in participating in screening after delivery). The belief that Pap smears are associated with increased rates of miscarriage or pre-term labour is a misconception that can contribute to unwillingness of women to have Pap smear taken in pregnancy. In such cases, a Pap smear can be postponed until the pregnancy is safely established. Similarly, every woman with unexplained bleeding in early pregnancy should have her cervix visualised via a speculum to ensure that unexpected malignancy is not the cause and should have her Pap smear repeated. It is important to recognize the pitfalls in cytology during pregnancy, described here.

Methods

Reports of absent endocervical component are more common in pregnancy. The use of a nylon or plastic brush and spatula in pregnant women has been shown e.g. in a Cochrane review to provide the highest rate of adequate smears. The plastic Cervex brush used under direct vision is another appropriate tool, whereas the use of Cytobrush is not recommended after 10 weeks of pregnancy. LBC is indicated if the smear is contaminated with mucus, which is often the case during pregnancy.

Results

In pregnancy, the cervix undergoes both glandular and stromal changes, similar to those occurring in the endometrium. The endocervical glands become hyperplastic and result in a polypoid protrusion and increase in the tunnel clusters, and/or microglandular hyperplasia. These hypersecretory cells contribute to the thick mucous plug, sealing off the endometrial cavity from the vagina, with a substantial amount of immature squamous metaplasia as the result. The stroma can also undergo focal or massive decidualization, which could be the cause of vaginal spotting in pregnancy. Occasionally the glandular cells exhibit nuclear clearing and can also undergo Arias-Stella reaction.

Perinuclear halos induced by glycogen as well as squamous-like syncytiotrophoblasts could mimic HPV. Endocervical cells with multi-nucleation or nuclear clearing could mimic herpes HSV infection. Cytotrophoblasts or degenerated decidual cells could mimic HGSIL. Despite the caution required in this population, dysplastic changes should not be underestimated.

Conclusion

For cytologists, it is essential to be familiar with the pregnancy-related changes to avoid misinterpretations. If, however, a high grade abnormality is confirmed, the management during pregnancy should follow the same guidelines as for the non-pregnant woman.

CS 04-03

Colposcopy and pregnancy

M. Roy

Laval University (Canada)

Background / Objectives

Colposcopy is mandatory when a significant abnormal pap smear is indicated in pregnant patient. The main purpose of Colposcopy during pregnancy is to rule out invasive cervical cancer. But one has to remember that this procedure is more difficult in pregnancy due to the physiologic changes: Hyper vascularization and congestion, cervical mucus, redundant vaginal walls, metaplasia, decidual changes. Inflammation, exaggeration of patterns and abnormal vascularisation are the most difficult evaluation for colposcopists, especially decidual "lesions". In order to avoid unnecessary colposcopic evaluation, the Society of Canadian Colposcopists (SCC) recommend: "Women with an ASC-US or LSIL test result during pregnancy should have repeat cytology testing at 3 months post pregnancy, rather than colposcopy", "Patients with CIN2+, ASC-H or AGC should be referred for colposcopy within 4 weeks", and endocervical curettage (SCC) should not be performed during pregnancy. When a CIN2+ is suspected, a biopsy is indicated. If the biopsy confirms a HSIL, a treatment is delayed 2 to 4 months after delivery. When an invasive cancer is suspected after a biopsy, a "partial" conisation can be done. In summary: Colposcopy is more difficult, so do not hesitate to ask for an expert consultation, a biopsy is not always necessary, if a « cone » is indicated, do a « directed excision ».

CS 04-04

CIN 2-3 TREATMENT IN PREGNANCY

E. Siegler¹, O. Lavie², Z. Vaknin³, A. Amit⁴, R. Auslander², Z. Blumenfeld⁴

¹Carmel Medical Center, Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa. (Israel), ²Carmel Medical Center, Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa (Israel), ³Assaf Harofe Medical Center, Sackler Faculty of Medicine, Tel Aviv University Tel Aviv (Israel), ⁴Rambam Medical Center, Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa (Israel)

Background / Objectives

Cervical Intraepithelial Neoplasia 2-3 is considered a premalignant lesion but during pregnancy the consensus guidelines are to treat it only if invasive cancer is suspected. The aim of this study is to describe our experience with women diagnosed with CIN 2-3 during pregnancy. We summarize data from the literature about the risk of CIN 2-3 progressing to invasive cancer during pregnancy and the safety of LLETZ during the first trimester.

Methods

The aim of the present study was to describe our experience with pregnant women diagnosed with CIN 2-3 that were followed up and treated after delivery and women who had LLETZ performed during the first 15 weeks of pregnancy. 84 pregnant women were diagnosed with CIN 2-3 between January 2006 and December 2015. 43 women were followed and 41 had LLETZ. We summarize the literature about the outcome of CIN 2-3 during pregnancy and information about excisional treatments performed during the first 15 weeks of pregnancy.

Results

43 women were followed up and the final pathological diagnosis was: 3 women (7.5%) cervical cancer, 26 (65%) had CIN 2-3 and 11 (27.5%) had CIN 1 or normal histology. Two women are still pregnant, two women had spontaneous abortion and one woman did not return for evaluation after delivery. Of the 41 women who underwent LLETZ during the first 15 weeks invasive cancer was diagnosed in 2 women (4.9%), CIN 2-3 in 36 women (87.9%) and 3 (7.3%) women had CIN 1 or normal histology. Severe bleeding needing suture occurred in one woman, minor bleeding occurred in two women and one woman had cervical cerclage at 21 weeks. In 7 women the LLETZ was performed together with termination of the pregnancy or treatment of missed abortion which was

diagnosed before the LLETZ .34 women continued their pregnancy, 31(91.2%) of them had term deliveries, two (5.9%) had late premature deliveries (at 34and 36 weeks), one woman had early missed abortion.Invasive cancer was the final diagnosis in 5 women (6.2%) of the 81 women that had completed the evaluation for CIN 2-3 during pregnancy.Summarizing 16 articles about 656 women diagnosed with CIN 2-3 during pregnancy the final diagnosis was invasive cancer in 45(6.9%).8 authors describe excisional treatments during the first 15-18 weeks of pregnancy with minimal complications, and term delivery in 90% of the women.

Conclusion

According to the presented data the LLETZ procedure during the first 15 weeks of pregnancy appears to be safe and has the advantage of diagnosing or preventing cervical cancer in 6.9% of the cases.Based on these results, we believe it is time to reconsider the indications and contraindications of CIN 2-3 treatment during the first 15 weeks of pregnancy.

References

Siegler E, Amit A, Lavie O, Auslender R, Mackuli L, Weissman A. Cervical intraepithelial neoplasia 2, 3 in pregnancy: time to consider loop cone excision in the first trimester of pregnancy?J. Low Genit Tract Dis. 2014; 18:162-8.

CS 04-05

CIN and reproductive morbidity: is it the treatment or is it CIN?

M. Kyrgiou

Imperial College London (United kingdom)

Conclusion

Since the first documentation of the reproductive risk associated with treatment almost a decade ago, more than 50 observational studies have been published confirming or disputing these associations; some of these reporting data from large population-based datasets. Individual attempts to synthesize parts of this rapidly evolving evidence base in small systematic reviews and meta-analyses reached contradictory conclusions and initiated debates and confusion within the scientific community.

Media publicity has heightened public awareness that treatment for cervical precancer is associated with an increased reproductive morbidity. There has been a substantial increase in enquiries from patients and clinicians on the risks associated with different treatment techniques and cone depths, and as to how this risk may be managed and prevented. With a rapidly evolving evidence base and lack of a robust synthesis of the published literature, these questions are becoming increasingly difficult to answer.

We recently conducted a series of systematic reviews and meta-analyses to explore the impact that CIN treatment on reproductive outcomes and to explore how this risk may be modified by the cone depth and comparison group. There was no evidence that fertility was affected after CIN treatment, although the risk of mid-trimester miscarriages was substantially higher. We also found that all local cervical treatments (excisional or ablative) increase the risk of preterm birth and adverse sequelae in a subsequent pregnancy. The magnitude of the effect of treatment was higher for more radical techniques and for excision rather than ablation. Multiple conisations increased four-fold the risk of preterm birth as compared to untreated controls. Subgroup analyses clearly demonstrated that the risk of preterm birth directly correlates to the cone dimensions (depth/volume) and progressively increases with increasing cone depth. Although the risk was increased even for excisions measuring less than 10mm in depth, this was almost two-fold higher for excisions of more than 10mm, three-fold higher for more than 15/17mm and almost five-fold higher for excisions exceeding 20mm in depth. We also found that although women with CIN have a significantly higher baseline risk of prematurity as compared to the general population, cervical treatment and particularly long cones further increase that risk.

The underlying mechanism is unclear; hypotheses include immunomodulation relating to HPV infection affecting parturition pathways, and acquired 'mechanical weakness' secondary to loss of cervical tissue. Future research should explore different possible aetiologies.

CS 05-01

A risk based approach to colposcopy and biopsy

N. Wentzensen

**Division of Cancer Epidemiology and Genetics National Cancer Institute -
Bethesda (United States of America)**

Background / Objectives

There is an ongoing debate about the best approach to colposcopy-biopsy to follow up positive cervical cancer screening results. Proposed strategies range from taking a biopsy only from the worst appearing lesion to taking four-quadrant biopsies irrespective of colposcopy impression. While these strategies are often discussed as a uniform approach for the entire colposcopy population, women referred to colposcopy have a wide spectrum of underlying risk of precancer that could influence colposcopic practice and management. For example, at a very low risk of precancer, taking multiple biopsies and targeting normal appearing cervix may not be necessary. At a very high risk of precancer, conversely, immediate treatment may be justified independent of the biopsy outcome.

Analyses of risk-strata based on different combinations of HPV status, cytology, p16/Ki-67, and colposcopic impression in women undergoing colposcopy in the Biopsy Study showed that combinations of test results from primary screening and triage together with colposcopic impression can be used to guide colposcopic practice. Analyses in other studies are currently underway and will be presented at the meeting. Developing risk-based guidelines for colposcopy and biopsy will be important to anticipate changes of current colposcopy populations related to HPV vaccination and HPV-based screening.

CS 05-02

EXCISION SHOULD ALWAYS BE PERFORMED UNDER DIRECT BINOCULAR COLPOSCOPIC VISION

X. Carcopino

Department of Obstetrics and Gynaecology Assistance Publique des Hôpitaux de Marseille (APHM) Hôpital Nord, Chemin des Bourrely, 13015 Marseille, France (France)

Background / Objectives

The risk of subsequent premature delivery is known to be related to the depth of excision, and it is significantly increased with the cone depth. However, there is growing evidence suggesting that even more so than the depth, the volume of the specimen could particularly be associated with the risk of subsequent premature delivery. Thus, when performing a LLETZ, every effort should be made to minimize not only the depth of the specimen, but also the volume, while obtaining negative margins, in order to achieve the exact balance between the highest efficacy of treatment and minimal pregnancy-related morbidity. The aim was to assess whether direct colposcopic vision (DCV) of the cervix during large loop excision of the transformation zone (LLETZ) is associated with a decrease in the volume and dimensions of specimens, or affects margin status at histology.

Methods

A prospective multicenter observational study of 216 women who underwent LLETZ for grade 2-3 cervical intraepithelial neoplasia (CIN) was conducted. The volume and dimensions (circumference, length and thickness) of the surgical specimens were measured before fixation. Data were compared according to the use of colposcopy during LLETZ. Three groups were considered: LLETZ performed without colposcopy (n=91), LLETZ performed immediately after colposcopy (n=51) and LLETZ performed under DCV (n=74).

Results

Patient characteristics were comparable with regards to age, parity, history of excision, indication of the procedure, and the size of the cervix. We found a significant decrease in all dimensions of the specimens obtained under DCV ($p < .001$). Margin status was not affected. After adjusting for confounders, the mean volumes were significantly lower in the DCV group (adjusted mean difference -0.66mL, 95%CI -1.17 to -0.14). The probability that negative margins would be achieved together

with the attainment of a volume below 5mL and a thickness below 10mm was the highest in the DCV group (adjusted OR 2.80, 95%CI 1.13 to 6.90).

Conclusion

DCV is associated with a significant decrease in the volume and in all dimensions of LLETZ specimens with no compromise in the margin status. By allowing for the precise location of both the upper and lateral limits of the abnormal transformation zone, colposcopy is likely to be the ideal tool for the optimization of LLETZ procedure.

CS 05-03

Challenges of colposcopy in non-cytology based screening program

P. Basu

Screening Group, International Agency for Research on Cancer (France)

Background / Objectives

Several countries across the globe have introduced cervical cancer screening program based on human papillomavirus (HPV) detection test or visual inspection with acetic acid (VIA) test and many more are contemplating to do so. Both the tests score over cytology in test sensitivity and certain logistic conveniences. However, specificity of either of the tests is mediocre and triaging of the test positives with colposcopy leads to large number of false positive diagnoses. In cytology screening prior knowledge of the grade of cytological abnormality guides the colposcopist to arrive at a diagnosis, an advantage that is absent in HPV or VIA based screening. As a consequence, the agreement between the colposcopy and the histopathology diagnoses is poor when colposcopy is performed on HPV or VIA positive women and the specificity is significantly reduced. Colposcopy tends to over-diagnose lesions and treatment based only on colposcopy diagnosis can potentially lead to significant number of over-treatments. Cytology triaging of the HPV positive women is the standard of care in the developed countries, which avoids the problems of false positive diagnosis or over-treatment to a great extent. However, most of the developing countries will have to depend on stand-alone HPV or VIA test in absence of quality cytology facilities and colposcopy will continue to be challenging.

In some studies random biopsies from cervix of HPV positive women without any colposcopically apparent lesion detected significant number of high grade lesions. Some authors put forward the concept of 'thin' CIN to account for the non-visualization of the lesions though this theory is debatable. It is more likely that the colposcopists failed to recognize small lesions or erosions.

HPV test is likely to detect more glandular lesions. In cytology screening the morphological diagnosis of a glandular abnormality guides the colposcopist to evaluate the cases adequately. This advantage will not be available in HPV detection based programs and the colposcopy may miss glandular lesions.

Conclusion

Colposcopy on women with abnormal cytology has been practiced widely for decades and as a result the management algorithms for different case scenarios are well standardized. Practice of colposcopy on HPV or VIA positive women is not yet standardized with certain unsettled

management issues e.g. management of screen positive women with type-3 transformation zone or of low grade lesions extending inside the canal.

There is an urgent need for a broad consensus on quality indicators for colposcopy as the same quality indicators and standards may not be applicable when colposcopy is performed on HPV or VIA positive women.

CS 06-01 POPULATION SCREENING PROGRAMME FOR CERVICAL CANCER SCREENING BASED ON HPV-m RNA PRIMARY TEST

T. Maggino

Director Obst. Gyn Dept. - Dell'Angelo Hospital Venice (Italy)

Background / Objectives

Following the results of Italian NTCC study, several pilot projects were started in Italy aiming to test feasibility and performance of HPV- test as primary screening in cervical cancer. In October 2011 the Local Health Authority of Venice (ULSS 12 – veneto Region) started a pilot study with HPV-m RNA test as primary screening test for the target population (women aged 25-64 years) of the local population screening. The test consists of APTINA HPV ASSAY (test for detection of HR-HPV E6/E7 m RNA; Hologic S. Diego, CA).

Methods

The study was approved by the local ethic-committee and a clear informed consent was sent to the women together with the invitation to perform the test. The screening algorithm includes samples collection with Thin-Prep transport device; samples were first processed for HPV-mRNA (Aptima); women with negative HPV were referred to a new screening test after three years; women with positive HPV test had a cytological Pap-test triage and invited to colposcopy if PAP >_ASCUS. Women with positive HPV test but negative PAP were referred to repeat HPV-test after one year and colposcopy if persistent positive.

Conclusion

The presentation will present results of the study for women enrolled in the period October 2011- May 2014 end followed until May 2015. Indications which will be discussed are: positivity at HPV-m RNA; referral rate to colposcopy; positive predictive value for CIN 2+ at colposcopy and biopsy; detection rate of CIN 2+.

Conflicts of interests: none

Funding: no funding was obtained for this study.

CS 06-03

Treatment Modalities of Preinvasive Diseases and Fertility Saving Surgeries in Gynaecological Cancers

A. Rodolakis

ATHENS UNIVERSITY, GREECE (Greece)

Background / Objectives

An increasing number of Gynecologic malignancies arise in premenopausal childbearing age for many women delaying conception. Fertility-sparing procedures for cervical carcinoma patients are the excisional conization of the cervix and the Radical Trachelectomy (RT). Nearly 50% of women younger than 40 years of age, eligible for surgical management, may be candidate for those conservative procedures.

Methods

Cervical conization is an effective treatment modality for preinvasive intraepithelial neoplasia and for FIGO stage IA1 cervical cancers meaning a ≤ 3 mm depth of invasion and horizontal spread ≤ 7 mm with no vascular or lymphatic invasion and negative resection margins. For stromal invasion more than 3mm and < 5 mm with spread ≤ 7 mm, stage IA2, cervical conization can locally control the disease. In stage IA2 nodal metastasis is 5-8%, thus lymphadenectomy is mandatory. For FIGO stage IA1 with lymphovascular space involvement, IA2 and IB1 tumors ≤ 2 cm limited to the cervix without evidence of lymph node metastasis, radical trachelectomy and pelvic lymphadenectomy is the optimal approach. The procedure comprises the removal of the cervix on a radical approach along with the paracervical tissue and the upper vagina by using the same technique as in a Schauta vaginal or Wertheim abdominal radical hysterectomy. As the first part of the procedure a pelvic lymph-node dissection is performed laparoscopically or as open procedure. An upper cervical or isthmic cerclage by using no 1 nylon suture is placed after the trachelectomy to help prevent possible cervical incompetence. A preoperative assessment with Magnetic MRI is used preoperatively in order to assess tumor diameter, cervical stroma infiltration and parametrial invasion.

Results

Lesion size is the most important risk factor for recurrence and tumors greater than 2 cm represent a significant increase in the risk of recurrence. The parametrial resection performed with the usual radical vaginal trachelectomy correlates to a class II radical abdominal hysterectomy, thus the procedure seems to be safe in patients with small < 2cm tumors. Radical Abdominal Trachelectomy with pelvic lymphadenectomy represents an alternative fertility-sparing approach for treating women with stage IB1 lesions with tumor diameter of 2-4cm. The radical abdominal trachelectomy may result in wider parametrial resection (more than 50% greater) than in radical vaginal trachelectomies.

Conclusion

Cervical conization and radical trachelectomy has similar oncologic outcomes to traditional radical approaches, and should be considered as alternative treatment options for young patients with early cervical cancer who wish to preserve their fertility.

CS 06-04

Ovarian and endometrial cancer screening

R. Manchanda

Barts Cancer Institute, Queen Mary University of London (United kingdom)

Two large prospective randomised trials have evaluated the impact of ovarian cancer screening in a low-risk general population on mortality. The PLCO trial (78126 women) used an absolute Ca125 value and TVUS as the screening intervention. The UKCTOCS study randomised 202638 women (1:1:2) to multimodal (using the risk-of-ovarian-cancer-algorithm (ROCA)) or TVUS based screening and controls. The use of a longitudinal-Ca125 ROCA analysis, strict call recall system as well as tight protocol-driven centralised co-ordination and management were unique to UKCTOCS. The PLCO study found no mortality benefit and a high 15% serious complication rate from surgical evaluation. The UKCTOCS study reported a 84%-85.9% sensitivity, 99.8% specificity and an acceptable PPV of 2.7 surgeries/cancer diagnosis. The complication rate was 3%. The authors reported a stage shift of 14% (40% vs 26%) \leq Stage-3a disease which is extremely noteworthy. The primary Cox regression analysis indicated a 15% non-significant mortality benefit with multimodal and 11% with US screening. However, interestingly the post-hoc analysis found a potential statistically significant delayed mortality benefit of 23% at >7 years post-randomisation. Unfortunately, the pre-specified UKCTOCS analysis plan did not account for any statistical adjustment for a potential delayed effect on mortality. Further follow-up of the cohort is being undertaken to confirm this. This is essential to confirm whether a stage-shift with lower volume disease and higher R⁰ cyto-reduction rates can translate into a mortality benefit.

Unlike high risk women (e.g. Lynch Syndrome), screening for endometrial cancer is not currently recommended in the low risk population as most women are symptomatic and present with early stage disease. Given increasing obesity and rising population incidence, this becoming an area of increasing concern. A nested case-control analysis within the UKCTOCS TVUS cohort of 37078 women shows that. The optimum cut-off for endometrial thickness (ET)=5.15 mm, with a RR=25.2 (CI 16.5,38.5). An ET=10mm, gives a sensitivity=54.1% (CI: 45.3,62.8) and leads to 17 diagnostic interventions/case diagnosed. An ET=5mm increases sensitivity(80.5%; CI:72.7,86.8) but requires 56 procedures/case diagnosed. Screening only the top quartile of the population (responsible for 40% cancers) yields a sensitivity=84.3% (CI:71.4,93.0) and specificity=89.9% (CI:89.3,90.5) for endometrial cancer using a cut off 6.75mm. Better identification of a higher risk category/sub-population may improve screening performance in asymptomatic women.

WACC I-02

HPV vaccination in Colombia

R. Murillo ¹, D. García ²

¹IARC (France), ²Ministerio de Salud (Colombia)

Background / Objectives

In 2012 HPV vaccination started in Colombia for girls 9 years and older in 4th school grade. The initial scheme used the quadrivalent vaccine at 0, 2, and 6 months, but in 2013, the vaccination schedule was modified to 0, 6, 60 and vaccination extended to all girls 9 years and older from 4th to 11th school grades. In June 2014 an outbreak was reported in a small village in the north of the country as possibly related with the HPV vaccination program.

Methods

The Colombian National Institute of Health carried out the corresponding research and surveillance according to established algorithms in addition to the medical examination and clinical investigation for all reported cases.

Results

During 5 months, 509 girls and 8 boys reported different symptoms including mainly (but not limited to) head ache, paresthesia, chest pain, dizziness, and fainting; all symptoms were associated by parents and children with a shot of HPV vaccine. Apart from the reported symptoms no additional alterations were observed in medical examinations (including lab, diagnostic imaging, and electrophysiological tests). No reports were received from other populations in the country and no relation with vaccine lot released was observed. The study did not find any relationship with the HPV vaccination program or any other possible source of the outbreak. HPV vaccination coverage in the country reached 89.2% of school girls in 2012 and 76.0% in 2013 (third and second doses respectively), after a massive diffusion of the outbreak episode in 2014 the vaccination coverage drop down to 21.2% in the country.

Conclusion

The role of the media and a proper response to massive reactions to HPV vaccination are essential to safeguard achievements of vaccination programs and women's health

WACC I-04

THE JAPANESE HPV VACCINE TRAGEDY: WHAT LESSONS CAN BE LEARNT?

S. Hanley

Hokkaido University Graduate School of Medicine, Department of Women's Health Medicine (Japan)

Background / Objectives

Two months after introducing the HPV vaccine into the National Immunization Programme, the Japanese Ministry of Health, Labour, and Welfare (MHLW) suspended proactive recommendations for the vaccine due to reports of adverse events following immunization (AEFI) in the media. Despite the Vaccine Adverse Reactions Review Committee repeatedly concluding no evidence exists to suggest a causal association between the HPV vaccine and reported AEFI, uptake rates plummeting from >70% to <1%, a study of 70,000 vaccinated and unvaccinated women in Nagoya showing no statistical association between reported symptoms and the HPV vaccine and Japan being criticized by GACVS for its policy decisions, the MHLW has, to date, failed to reinstate proactive recommendations. This paper reviews the political, socio-cultural and health system factors that have led to such an unprecedented situation.

Methods

Government data and offline, online/social media are examined.

Results

Firstly, the HPV vaccine was introduced without any forward planning or systematic investigation of parental concerns. Most educational materials were developed by pharmaceutical companies, none by the government, leading to an atmosphere of mistrust among the media and the public in general. Additionally, lack of precise epidemiological data based on a national surveillance system has meant the MHLW had not been able to carry out observed versus expected analyses to provide the public with incidence rates of purported side effects before and after introduction of the vaccine. Secondly, the MHLW has been hit with a better organized anti-vaccination movement who have gained control of the narrative through offline, online/social media, sensational video clips and highly publicized events. Furthermore, the MHLW has never refuted or criticized false media reports. Finally, the involvement of a few senior physicians proposing a new condition, HPV Vaccine Associated Neuropathic Syndrome (HANS), along with the involvement of a drug disasters monitoring NGO, who brought the case of HIV-contaminated blood products the most notorious drug scandal in Japan, to reconciliation in 1996, has meant most major Japanese media outlets present the HPV vaccine

'victims' in the same light as the hemophiliacs who developed AIDS after receiving the tainted blood products.

Conclusion

No vaccine safety signal has been recorded in Japan. Instead, individuals who have the misfortune to be unwell with rare or difficult to treat disorders have been encouraged by antivaccination advocates to blame the HPV vaccine, especially in an unrestrained media environment and with little reassurance and systematic addressing of these events by the government.

WACC I-06

ANALYSIS OF INFLUENCES AFTER SUSPENSION OF PROACTIVE RECOMMENDATION FOR HPV VACCINATION IN JAPAN

E. Miyagi¹, Y. Motoki¹, Y. Suzuki², M. Asai-Sato², A. Sukegawa¹, T. Egawa-Takata³, Y. Ueda³, R. Kudo⁴, M. Sekine⁴, T. Enomoto⁴, S. Hanley⁵, R. Konno⁶

¹Dept. of Obstetrics and Gynecology, Yokohama City University Graduate School of Medicine (Japan), ²Dept. of Obstetrics and Gynecology, Yokohama City University Graduate School of Medicine (Japan), ³Dept. of Obstetrics and Gynecology, Osaka University Graduate School of Medicine (Japan), ⁴Dept. of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences (Japan), ⁵Dept. of Women's Health Medicine, Hokkaido University, Sapporo (Japan), ⁶Dept. of Obstetrics and Gynecology, Jichi Medical University Saitama Medical Center, Saitama (Japan)

Background / Objectives

Cervical cancer incidence and mortality among young Japanese women is increasing. Although HPV vaccination was implemented as a part of the national vaccine program from April 2013, Japanese governmental authorities suspended proactive recommendation of HPV vaccines two months later in July 2013, because of repeated media reports of adverse events after vaccination. We analyzed scientific reports concerning HPV vaccination in Japan published thereafter.

Methods

PubMed was used to find related articles. We divided about 30 topics into related categories (epidemiological topics, socio-medical studies, adverse events, etc.) and analyzed the content.

Results

Epidemiological topics focused on the low uptakes of HPV vaccination for the targeted age-group after suspension of recommendations, and stressed the necessity of HPV vaccination in Japan considering low-uptakes of cervical cancer screening. Studies using a socio-medical approach revealed the enormous influence of the media and governmental attitudes on general citizens' decision not to vaccinate adolescent girls against HPV. Reports concerning adverse events after HPV vaccination in Japan described various symptoms including Postural Orthostatic Tachycardia Syndrome (POTS) and/or Chronic Regional Pain Syndrome (CRPS). However, some investigator

groups claimed that causality had not been proved at all. Related academics both within and outside Japan expressed their determinate support for HPV vaccines.

Conclusion

Restart of governmental recommendations for HPV vaccines is considered to be a minimum requirement to change the present negative recognition of HPV vaccines in Japan. In addition, risk communication among health care providers, academics, citizens and the media are indispensable for the progress of cervical cancer prevention in Japan as a developed country.

References

Motoki Y, Miyagi E, et al. Increasing trends in cervical cancer mortality among young Japanese women below the age of 50 years: an analysis using the Kanagawa population-based Cancer Registry, 1975-2012. *Cancer Epidemiol.* 2015; 39: 700-6.

Hanley SJ1, et al. HPV vaccination crisis in Japan. *Lancet.* 2015; 27; 385:2571.

Egawa-Takata T, Ueda Y, Sekine M, Enomoto T, et al. Survey of Japanese mothers of daughters eligible for human papillomavirus vaccination on attitudes about media reports of adverse events and the suspension of governmental recommendation for vaccination. *J Obstet Gynaecol Res.* 2015, 41; 1965-71.

Kinoshita T, Ikeda S, et al. Peripheral sympathetic nerve dysfunction in adolescent Japanese girls following immunization with the human papillomavirus vaccine. *Intern Med.* 2014; 53: 2185-200.

WACC I-07

Sex and Intimacy after advanced cervical cancer

A. Hicks

The Hicks Foundation- USA nonprofit for Education on HPV cervical cancer (United States of America)

Background / Objectives

"I Wish She Had Told Me..." Perspectives on Sexuality and Intimacy After a Cervical Cancer Diagnosis

1. Identify common barriers to communication between the Cervical Cancer Survivor and her partner.
2. strategies Cervical Cancer survivors and their partners can use to open and/or improve communication related to sexuality and intimacy.

Methods

This is not a science paper or reseach. This is my personal experience and informed awareness about sexual dysfunction after cervical and other cancers.

1. Common barries- Body image after surgery, menopause, depression, loss of sex drive and sexual desire, not feeling as wanted, fear of rejection, not knowing your own sexual needs as they are new.
2. Strategies to improve communication: talk openly, must practice masturbation and own personal sexual abilities and desires so that you can share with partner, learn about sexual tools, lubricants, and toys, learn about other ways to improve intimacy other then sex. Other forms of touch and closeness. Developing trust again. Therapy and counseling.

Conclusion

This will be an open oral presentation. I will discuss each of the many possible barriers to communication and sexual dysfunction. and give examples of how to achieve growth in both from my own personal experience and years as a womens heath advocate. Secondly I will discuss the strategies to increas communication and intimacy for couples and for personal increased self love and sexual happiness. This is an issue that I fear is never discussed and I have performed this presenation before and it was highly recommended. If there is a MD who would like to go over the anatomy of female genitalia and what is the damages of surgery,menopause and lack of sexual intercourse play on how the sex drice decreases and function declines and it is our duty to keep it up and health.

WACC II-02

MALE MEDICAL STUDENT' INTEREST AND PERCEPTIONS OF OBSTETRICS AND GYNECOLOGY

W. Alsarhani, S. Albogami, M. Alotaibi, Z. Alamari, S. Alahmari, M. Bawazeer

King Saud bin Abdulaziz University for Health Sciences (Saudi arabia)

Background / Objectives

Obstetrics and gynecology (OBGYN) is currently one of the professions females have just dominated in many countries of the world. With fewer males choosing OBGYN there is failure to fill all the residency positions in Saudi Arabia. The objective of the study was to find out the perceptions and interests of final year male medical students in OBGYN.

Methods

This study was survey based cross sectional study on final year male medical students in King Saud bin Abdulaziz University for Health Sciences (KSAUHS), Riyadh Saudi Arabia in February 2016. The questionnaire was validated, and a Cronbach alfa reliability test was 0.778.

Results

A total of 84 questionnaires were returned with 92 % response rate. 18% of all male medical students think OBGYN is a socially accepted specialty, and 32% think it is a boring specialty. Only 4 students (4.8%) were interested in OBGYN. Those interested perceived OBGYN as an advanced specialty ($P= 0.036$). Half of the students interested were married compared to only 5% marital status in those not interested in OBGYN ($P=0.001$). 88% of students who haven't in rotated in OBGYN think it is boring, while 52% of students who rotated in OBGYN don't think so ($P=0.01$).

Conclusion

The number of male medical students interested in OBGYN isn't increasing. In order to increase the number of obstetricians and gynecologists and improve health care, public awareness about the fact OBGYN needs male physicians should be raised.

WACC II-03

BARRIERS TO CERVICAL CANCER SCREENING AMONG ROMA-WOMEN IN ROMANIA: A QUALITATIVE STUDY

T. Andreassen¹, E. Weiderpass¹, O. Suteu², F. Nicula³, A. Tincu⁴, M. Bumbu³, A. Itu⁵, K. Moen⁶

¹Cancer Registry (Norway), ²Prof. Dr. Ion Chiricuta, Institute of Oncology and Iuliu Hatieganu, University of Medicine and Pharmacy, Cluj-Napoca (Romania), ³Prof. Dr. Ion Chiricuta, Institute of Oncology, Cluj-Napoca (Romania), ⁴Iuliu Hatieganu, University of Medicine and Pharmacy, Cluj-Napoca (Romania), ⁵Prof. Dr. Ion Chiricuta, Institute of Oncology, Cluj-Napoca (Norway), ⁶University of Oslo (Norway)

Background / Objectives

Romania has Europe's highest incidence and mortality of cervical cancer. A national screening programme targeting women aged 25 to 64 with conventional cytology 5 yearly was started in 2012. Primary sample takers are gynaecologists (66%) and GP's (34%). With referral from a GP, testing is free, as is treatment, if cancer is diagnosed. However, re-testing and follow-up of precancerous lesions is not covered. By 2015, 20% of eligible women in Cluj County had participated in the programme, but women without health insurance, from rural areas, and belonging to ethnic minorities, seldom attend screening. We studied possible barriers to participation among Roma.

Methods

In 2015, we carried out participant observation, qualitative interviewing, and focus group interviews among Roma in Cluj and Bucharest. Qualitative content analysis was carried out.

Results

A fundamental barrier to participation was that the screening programme was almost entirely unknown among Roma women. Moreover, when told that the programme was indeed in existence and that primary testing was free, women assumed it was not meant for them. This impression arose from knowledge of women who had taken Pap smears and had had to pay for them. The ubiquity of this impression raise questions about how well known the programme was among gynaecologist and GP's, and to what degree it was being executed according to intentions. While some had good knowledge about cervical cancer, a large majority had very limited insight into medical knowledge about the condition. Yet, almost everyone knew that cervical cancer is potentially serious, and it was

a widely shared view that screening would be a very good thing. "If people knew about this, everyone would participate," was the consensus in one focus group. However, that participation and follow-up should be free was considered extremely important. Most women were poor and did not have health insurance, and many worried they would not be able to afford care. Significant uncertainties also emerged about how a screening programme would engage with the Roma community as many had experienced discrimination in health care system and expressed doubts about whether healthcare workers in a screening programme would treat them well

Conclusion

There appeared to be no lack of interest in screening. However, there is a need for information about the programme and how it works, and for positive relation-building with Roma communities. Since familiarity with medical knowledge about cervical cancer was low, the program should make information on the condition widely available. To make re-testing and follow-up of precancerous lesions free, would seem to be important for screening success.

WACC II-04

The attitude of Hungarian male high-school students' concerning the HPV vaccine

B.C. Balla, A. Terebessy, P. Balázs

Semmelweis University, Department of Public Health (Hungary)

Background / Objectives

Cervical cancer, which is due to an infection from the human papillomavirus (HPV) is the eighth most common malignancy in Hungary. The virus in males, among many others, may cause genital warts, penile cancer or anal cancer. Throughout the country, 12-13-year-old girls receive the HPV vaccine as part of the school vaccination campaign since September 2014.

Our aim was to assess the knowledge of young Hungarian men regarding the HPV infection and cervical cancer, and to examine their attitude towards the HPV vaccine.

Methods

We conducted a cross sectional analysis between March 2013 and May 2014 with the distribution of questionnaires in the Hungarian capital in 19 randomly selected secondary schools among 530 senior male students, above the age of 18. The 54 multiple choice questions referred to their socio-demographic background, their lifestyle factors, their knowledge of HPV and cervical cancer and their attitude towards the HPV vaccine.

Results

Only 35.3% of the young men knew HPV was an STD, and only 3.5% was aware of the possibility of transmission via skin contact. The majority, 52.5% had already heard that the infection can cause cervical cancer, however less than 10% was able to relate it to other diseases. The risk factors of HPV infection was relatively unknown to the boys, only a third of them had heard of promiscuity as a risk factor. As for the HPV vaccine, 7.6% had already received it and further 6.8% would like to be vaccinated. 44.7% of the young men would have their future children vaccinated, while 24.5% remain indecisive for now. Approximately one out of four students would make the HPV vaccine compulsory (25.5%), 38.9% cannot decide right now. 56% trust the vaccine with doubts.

Conclusion

The knowledge of young men studying in Budapest regarding HPV and cervical cancer was poor. Their attitude toward the HPV vaccine was relatively positive.

WACC II-05

Do school requirements increase HPV vaccination coverage?

N. Brewer¹, J. Moss¹, P. Reiter², Y. Truong¹, B. Rimer¹

¹University of North Carolina (United States of America), ²Ohio State University (United States of America)

Background / Objectives

Suboptimal human papillomavirus (HPV) vaccination coverage in the United States is an ongoing crisis in cancer prevention. We assessed the impact of states' school-entry requirements for adolescent vaccination, focusing on HPV vaccination.

Methods

We categorized US states according to their 2007-2012 school-entry requirements for adolescent vaccination (tetanus, diphtheria, and pertussis (Tdap) booster; meningococcal; and HPV). The National Immunization Survey-Teen (2008-2012) provided physician-verified data for 99,921 adolescents that we used to calculate vaccination coverage (percent of 13- to 17-year-olds vaccinated), timeliness (percent of adolescents vaccinated by age 13), and seasonality (percent of doses administered in June, July, and August). We also assessed same-day ("concomitant") vaccination. HPV vaccination outcomes were among female adolescents only. We used longitudinal, weighted multivariable regressions to examine the impacts of vaccination requirements on these outcomes.

Results

Many states adopted Tdap booster (7-42 states) or meningococcal vaccination requirements (0-14 states), but very few adopted HPV vaccination requirements (0-2 states). Tdap and meningococcal vaccination requirements increased coverage for targeted vaccines (22-24% absolute higher coverage in states with versus without requirements), but HPV vaccination requirements did not (<1%). Tdap and meningococcal vaccination requirements also had spillover effects for HPV vaccines (4-8%). Tdap and meningococcal vaccination requirements were associated with increases in coverage, timeliness, and seasonality (all $p < .05$) for all targeted, spillover, and concomitant vaccinations.

Conclusion

School-entry requirements for HPV vaccination are ineffective in the US, but requirements for meningococcal vaccination could substantially improve HPV vaccination coverage. Policymakers and clinicians should anticipate and capitalize on changes in adolescent vaccination patterns that may arise after states adopt vaccination requirements.

WACC II-06

PARENTS' VIEWS OF INCLUDING BOYS IN THE HPV VACCINATION PROGRAMME

M. Gottvall¹, C. Stenhammar², M. Grandahl³

¹Department of Public Health and Medicine, The Red Cross University College, Stockholm / Department of Public Health and Caring Sciences, Uppsala University, Uppsala (Sweden), ²Department of Public Health and Caring Sciences, Uppsala University, Uppsala (Sweden), ³Department of Public Health and Caring Sciences / Department of Womens' and Childrens' Health, Uppsala University, Uppsala (Sweden)

Background / Objectives

The national HPV vaccination programme in Sweden only includes girls. Several other countries have included also boys in their programmes. There are currently discussions in Sweden about whether it is beneficial to also include boys in the programme.

The objective of the present study was to explore parents' views of extending the vaccination programme to also include HPV vaccination for young boys.

Methods

Interviews with parents (N=42) who were offered HPV vaccination for their 11–12 year old daughter. Interviews were recorded and transcribed verbatim. Data were then analysed with qualitative content analysis.

Results

The analysis resulted in five preliminary categories: Inequality in health services, Preference for gender equal vaccination, No reason to vaccinate boys against a female disease, Girls are more vulnerable, and Inequality in sexual and reproductive health responsibility. In the interviews, parents expressed low awareness about male HPV vaccination and the risk for boys to contract HPV infection and HPV-related cancer. However, several parents asked the question "If boys can spread this virus why shouldn't they be vaccinated?" Some parents believed girls were targets for a vaccine experiment and some parents questioned the safety and benefits of the vaccine since it was not offered to all children. On the other hand, parents also saw it as positive that girls' health was prioritized. Some parents were not willing to vaccinate their daughter because she was seen as too vulnerable but they were positive towards vaccinating their sons.

Conclusion

A vaccine offered only to girls may create vaccine hesitancy among parents. Including also boys in the national vaccination programme might be beneficial to improve parents' trust in the vaccine. This may lead to increased HPV vaccine coverage among both girls and boys eventually.

WACC II-07

PEDIATRICIAN COMMUNICATION ABOUT HPV VACCINATION: AN ANALYSIS OF RECORDED CONVERSATIONS

A. Kulkarni¹, L. Sturm², K. Donahue², M. Kasting², N. Brewer³, G. Zimet³

¹Merck & Co., Inc. (United States of America), ²Indiana University (United States of America), ³North Carolina University (United States of America)

Background / Objectives

U.S. HPV vaccination rates are lower than desired. Lack of a strong health care provider (HCP) recommendation is considered a primary reason for non-vaccination. We evaluated HCPs' conversations with parents of eligible children to evaluate communication strategies.

Methods

In 2013 19 U.S. pediatricians contributed 75 audio recordings of discussions with parents of 11-12 year old children about vaccination. HCPs, parents, and patients consented to the recordings but were unaware of the focus of the project. Recordings were transcribed and analyzed via qualitative data analysis.

Results

Of the 75 encounters, 22 (29%) resulted in same day HPV vaccination. Most communication about HPV vaccine involved mixed messages. It often was treated differently from other vaccines, with providers describing other vaccines as "required by school" but HPV vaccine as "optional". Many HCPs recommended delaying HPV vaccine even before a parent voiced a reaction. HCPs' gave as rationales for delay, that the child is "too young" (e.g., "I'm hoping he waits until high school to... interactionally have relationships, so we have plenty of time"). Some providers talked about a gradual introduction of the vaccine (e.g., "I usually mention that at this visit because at some point in the next 2 to 3 years, you're probably going to want to consider it for her"). Once HCPs endorsed delay of HPV vaccine, parents often agreed. Furthermore, when providers recommended the vaccine, but encountered parental hesitancy, they typically acquiesced immediately. In contrast, when effective communicators encountered a hesitant parent, they inquired about the source of concern (e.g., "So what kind of questions do you have about it that you're unsure?"). There were a few providers who discussed HPV vaccine much as they did other vaccines, adopting a matter-of-fact,

presumptive approach which included HPV vaccine in a list of vaccines for which the child was due: (e.g., “today she is getting...; today he is due for...”). Adopting an “I assume this will happen today” stance, they might say: “let’s get those started for you; it is 3 doses, you get one today; Everybody is getting HPV today.” Chi-Square tests indicated that mentioning delaying vaccination led to significantly lower HPV vaccine acceptance ($p < .001$), whereas using presumptive language led to higher acceptance ($p < .01$).

Conclusion

Few HCPs recommended HPV vaccine in a routine straight-forward manner, instead treating it different from other adolescent platform vaccines. Use of presumptive language and avoidance of mentioning delaying vaccination are important to successful timely administration of HPV vaccine.

WACC II-08

A SCHOOL-BASED EDUCATIONAL INTERVENTION CAN INCREASE ADOLESCENTS KNOWLEDGE AND AWARENESS ABOUT HPV

M. Grandahl¹, **A. Rosenblad**², **C. Stenhammar**³, **T. Tydén**³, **R. Westerling**³, **M. Larsson**⁴, **M. Oscarsson**⁵, **B. Andrae**⁶, **T. Dalianis**⁷, **T. Nevéus**⁴

¹Department of Public Health and Caring Sciences, Uppsala University, Uppsala; Department of Women's and Children's Health, Uppsala University, Uppsala (Sweden), ²Centre for Clinical Research Vasteras, Uppsala University, Central Hospital, Vasteras (Sweden), ³Department of Public Health and Caring Sciences, Uppsala University, Uppsala (Sweden), ⁴Department of Women's and Children's Health, Uppsala University, Uppsala (Sweden), ⁵Department of Public Health and Caring Sciences, Uppsala University, Uppsala; School of Health and Caring Sciences, Linnaeus University, Kalmar (Sweden), ⁶Centre for Research and Development, Uppsala University/Region of Gävleborg, Gävle (Sweden), ⁷Department of Oncology-Pathology, Karolinska Institutet, Karolinska University Hospital, Stockholm (Sweden)

Background / Objectives

Background: In Sweden HPV vaccination is offered to girls aged 10-12 years within the school-based vaccination program, while older girls (13-26 years) are offered the vaccine through the primary care. The vaccination rates are substantially lower (59%) among the catch-up group compared to the younger age group (82%). Adolescents have low awareness and knowledge about HPV, especially regarding cancer risks. The providers - school nurses - play a key role in providing such information. Upper secondary school students, aged 16 years, are by the school nurse offered a health interview, which includes a dialogue regarding their health, including sexual health. The health interview does however not include systematic information about HPV.

Objectives: To improve adolescents' knowledge and awareness about primary prevention of high risk HPV infection.

Methods

Methods: A cluster-randomised controlled trial among upper secondary schools (n=18) was performed. Schools were first randomised to an intervention or control group, after which individual classes were randomised. In total, 832 students, boys and girls aged 16 years attending theoretical or vocational programs were invited to participate. In the end, 741 (89.1%) students completed the

study. The intervention was based on the Health Belief Model (HBM). School nurses delivered 30 minutes of face-to-face structured information about HPV, including cancer risks and HPV prevention (i.e. condom use and HPV vaccination) to the intervention group. Students in both groups completed questionnaires at baseline and at follow-up after three months. The control group received standard treatment, i.e. the regular health interview with the school nurse. Generalized estimating equation analyses were used for examining the results of the intervention.

Results

Results: The intervention had positive effects on the adolescents' knowledge ($p < 0.001$), with a 0.582 higher score for the intervention group compared to the control group. There were no differences in knowledge due to sex ($p = 0.093$) or immigrant background ($p = 0.592$). The intervention also increased awareness ($p < 0.001$), with a 0.590 higher score for the intervention compared to the control. Again, there were no differences in awareness due to sex ($p = 0.183$) or immigrant background ($p = 0.319$).

Conclusion

Conclusions: The school-based intervention delivered by school nurses, had favourable effects on knowledge and awareness about primary prevention of HPV among adolescents aged 16 years.

References

Trial registration - ClinicalTrials.gov Identifier: NCT02280967

WACC II-09

HPV VACCINATION, SURVEILLANCE AND SOCIETY: MEETING THE NEEDS OF THE EUROPEAN INFLUX OF REFUGEES AND ASYLUM SEEKERS.

M. Heffernan, E. Holroyd

RMIT Univeristy (Australia)

Background / Objectives

Prophylactic HPV vaccines have reduced HPV morbidities but vaccine hesitancy prevails in many groups globally. The unprecedented European influx of refugees and asylum seekers presents challenges in meeting the objectives of the European Vaccine Action Plan 2015–2020¹, which urges culturally appropriate vaccination services and information. The disparate socio-cultural values of this influx of immigrants have implications for HPV vaccination programs. The complex factors that contribute to decision making are especially prevalent in immigrant populations where sexual health literacy is low or non-existent.

Methods

We report the findings from a study undertaken with East African-Australian communities in Melbourne that has implications for the related diaspora of European refugees. A community-partnership approach with multicultural agencies and a bi-lingual translator through focus group discussion explored the socio-cultural health, ecological and historical determinants contributing to HPV vaccine hesitancy and uptake.

Results

In Melbourne, the East African parent communities had no sexual health knowledge and perceptions of HPV and HPV vaccines. Multiple factors contributed to vaccine hesitancy including disparate sexuality norms. Redevelopment of public HPV information resources that incorporate health culture, social inclusion, and cultural continuity in a common first language enabled community-partnership engagement and empowerment. The increased knowledge of HPV factors among adult participants is expected to lead to intentions of screening in women and health checks in men.

Conclusion

If the current influx of refugees is to experience long-term well-being through their integration into European health and social systems, diverse sexuality norms will need to be reframed. This will require a rethink of current HPV vaccine education and prevention approaches to reduce the current cultural vacuum and insecurities.

References

1. World Health Organisation. "How to present the European Vaccine Action Plan 2015-2020 (EVAP) (http://www.euro.who.int/__data/assets/pdf_file/0020/253730/64wd15e_EVAP_140459.pdf) (Accessed 27th February 2016)

WACC II-10

THE EFFECT OF SOCIAL MEDIA CAMPAIGNS ON YOUNG WOMEN'S ATTENDANCE RATE TO CERVICAL CANCER SCREENING IN NORWAY

E. Jakobsen¹, S. Storhaug², I. Ross², L. Thorsen², A. Tropé¹

¹Cancer Registry of Norway (Norway), ²Norwegian Cancer Society (Norway)

Background / Objectives

Norway has a national screening program against cervical cancer, where all women 25-69 years are invited to take a screening test every third year. The overall coverage, and particularly among the youngest women, aged 25 to 29, is unsatisfactory. When the program started in 1995, the attendance was at its peak, at 73 percent. The coverage rate has decreased continuously since, to an all-time low in 2012, at 52 percent.

By connecting different actors concerned with the low coverage, the idea was to create a campaign using different media channels, but with particular emphasize on social media networks. Involved in the campaign were: The Norwegian Cancer society, Det Nye, a glossy magazine for young women and The Norwegian Cancer Registry.

Methods

A national campaign, #sjekkdeg ("get checked"), was launched in September 2015 after a young blogger diagnosed with cervical cancer started blogging about her disease in March 2015.

The campaign included: short films featuring young, female Norwegian celebrities, editorial pieces in the magazine Det Nye, press coverage in other mainstream media (more than 60 reports), blog posts (3,2 million views) and social media activity with the hashtag #sjekkdeg (more than 700 postings on Instagram). The number of registered screening tests, and 3.5 year screening coverage by age, were calculated from the national screening databases at the Cancer Registry of Norway.

Results

In 2015, the number of registered screening tests among women in the age group 25 to 29 increased by more than 6500 (13 percent) compared with the year before.

The total coverage for the years 2012-2014 among women aged 25 to 29 was 54.9 percent (Poisson exact 95 percent confidence interval from 54.6 to 55.3 percent). By the end of 2015, the 3.5 year coverage had increased significantly to 59.1percent (95 percent CI 58.7-59.4 percent)

Conclusion

Raised awareness on cervical cancer in Norway has contributed to increased attendance to the screening program over the latest years. It is also reasonable to think that a large proportion of the increase can be attributed to the #sjekkdeg-campaign in 2015. This campaign indicates that unconventional thinking can be useful, and that employing new media channels that reaches the target audience directly can affect the screening coverage.

The results from the first year of the campaign has ensured the parties in the collaboration that the work should continue, with new campaign periods.

WACC II-11

SOCIAL MOBILIZATION, ACCEPTABILITY AND CONSENT DURING HUMAN PAPILLOMAVIRUS VACCINATION IN LOW- AND MIDDLE-INCOME COUNTRIES

S. Kabakama¹, **K.E. Gallagher**², **N. Howard**³, **S. Mounier-Jack**³, **H.E.D. Burchett**³, **U.K. Griffiths**³, **M. Feletto**⁴, **D.S. Lamontagne**⁴, **D. Watson-Jones**⁵

¹Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania (Tanzania, united republic of), ²Clinical Research Department, London School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7H, UK & Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania (United kingdom), ³Department of Global Health and Development, London School of Hygiene and Tropical Medicine, Tavistock Place, London, WC1H 9SH, UK (United kingdom), ⁴PATH, Vaccine Access and Delivery, PO Box 900922, Seattle, WA, 98109, USA (United States of America), ⁵Clinical Research Department, London School of Hygiene and Tropical Medicine, Keppel St, London, WC1E 7H, UK & Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania (Tanzania, united republic of)

Background / Objectives

This study synthesizes experiences and lessons learnt around social mobilization, consent and acceptability from 55 HPV vaccine demonstration projects and 8 national programmes in 37 low and middle-income countries (LMIC) between January 2007 and January 2015.

Methods

The qualitative study design included: (i) a systematic review, in which 1,301 abstracts from five databases were screened and 41 publications included; (ii) soliciting 124 unpublished documents from governments and partner institutions; and (iii) conducting 27 key informant interviews. Data were extracted and analysed thematically.

Results

Almost all experiences framed mobilization messages around vaccine-induced protection from cervical cancer, rather than prevention of a sexually transmitted infection. Rumours were consistent

across world regions and largely focused on the effect of the vaccine on girls' fertility. Experiences emphasized that it was critical to address rumours as soon as they emerged. Interactive communication with parents was more likely to achieve high uptake than non-interactive messaging. Political and/or celebrity champions proved useful in mobilizing both girls and their parents. Acceptability was generally determined by personal knowledge of the vaccine's benefits, acceptance within the surrounding community, limited exposure to rumours and knowing where and when to go for vaccination.

Conclusion

Social mobilization strategies and factors influencing HPV vaccine acceptance are consistent across world regions and projects/programmes. Thus, further formative research may not be required. Countries introducing HPV vaccination or increasing coverage can learn from the available experiences, regardless of their economic status.

WACC II-12

HPV vaccination in Japan: early effectiveness and concerns

R. Konno¹, H. Konishi², D. Hotta³, E. Miyagi⁴, I. Arakawa⁵, T. Kakizoe²

¹Jichi Medical University Saitama Medical Center (Japan), ²Japan Cancer Society (Japan), ³Jichi Medical University Saitama Medical Center (Japan), ⁴Yokohama City University (Japan), ⁵Teikyo Heisei University (Japan)

Background / Objectives

Since June 14th 2013, the Japanese Ministry of Health Labour and Welfare (MHLW) has suspended recommendations for the HPV vaccination program. The first cohort of adolescent girls vaccinated with HPV vaccine against HPV 16 and 18 at age 16 yrs (birth year 1994), reached the Japanese screening age of 20 in 2014. These girls, along with girls born between 1995 and 1999, were vaccinated between November, 2011 and March, 2013 with public funding from specific budgets of the national and regional government. In April, 2013, a National Immunization Program started, but sensational media reports adversely affected it without any scientific proof. Japan has no linkage of screening and vaccination programs unlike in Australia, the UK, Nordic countries and Canada, etc. Thus, using data from the Japan Cancer Society is the only way to obtain national data to evaluate population-based effectiveness of HPV vaccination in Japan.

Methods

A part of nationwide data on cervical screening results for women aged 20 to 29yrs (a total of 31,890 subjects) in 2014 was obtained from 15 branches of the Japan Cancer Society. At screening, women had to fill in a questionnaire about HPV vaccine status and this data was used to analyze the relationship between vaccine status and incidence of CIN3.

Results

Among 31,890 women, 1,207 women were vaccinated and 30,483 women were unvaccinated. Overall 3.8% of the women had been vaccinated against HPV. In women aged 20 and 21 years, it was 32.3% and 7.5%, respectively. Among women vaccinated and unvaccinated, CIN 3 lesions were cumulatively detected in 1 case (0.08%) and 79 cases (0.26%) for 1 year, respectively. Thus, risk ratio on development of CIN 3 lesions was 0.31 [95%CI: 0.04-2.21], but not statistically significant.

Conclusion

This preliminary result may suggest the early impact of relatively effectiveness of HPV vaccination but further evaluation is warranted. It is necessary that the government and academia explains about the effectiveness and safety of the HPV vaccine based on scientific evidences with epidemiological surveillance. As GACVS has noted previously, policy decisions based on weak evidence, leading to lack of use of safe and effective vaccines, can result in real harm (1, 2).

References

1. http://www.who.int/vaccine_safety/committee/topics/hpv/Dec_2015/en/

2.

http://www.who.int/vaccine_safety/committee/topics/hpv/GACVS_Statement_HP_V12_Mar_2014.pdf

WACC II-13

The efficacy of HPV vaccine in Japanese women aged 20-21 years old

R. Kudo¹, **M. Sekine**¹, **M. Yamaguchi**¹, **S. Adachi**¹, **Y. Tanaka**², **A. Morimoto**², **T. Takata**², **A. Yagi**², **Y. Ueda**², **T. Enomoto**¹

¹1) Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Science (Japan), ²2) Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine (Japan)

Background / Objectives

Cervical cancer has been increasing rapidly in Japanese young women, however, administration of the human papillomavirus (HPV) vaccine decreased dramatically in Japan after extensive news of adverse vaccine events and suspension of the governmental recommendation for the vaccine. Our aim of this study is to clarify the effectiveness of HPV vaccination against HPV infection and cervical cancer in Japanese women.

Methods

Six hundreds eighty one Japanese women aged 20-21 years were enrolled in this study between April 2014 and January 2016 in Niigata, Japan. We obtained information of sexual behavior and HPV vaccination in 584 girls (85.7% of all registrants). We investigated Pap smear and HPV infection screening(QIAGEN HC II) in all registrants, then if HPV was positive, we added HPV typing(MEBGEN TM HPV kit). A Fisher's exact test and chi-square test was used in this statistical analysis.

Results

There were 247 cases (42.3%) in HPV vaccine-exposed (vaccine+) group and 337 cases (57.7%) in HPV vaccine-unexposed (vaccine-) group. The HPV infection rate was significantly higher as sexual debut age was younger and number of sexual partners increased, respectively ($p < 0.0001$). However, the HPV vaccination rate tended to decrease as sexual behavior became active. No one had HPV 16 and 18 infection in vaccine+ group, on the other hand, 6 women ($6/337=1.7\%$) had HPV 16 and 18 infection in vaccine- group. This difference was statistically significant ($p=0.042$). The HPV infection rate of vaccine+ group was not significantly lower than that of vaccine- group in early sexual debut age women (<15 years old).

Conclusion

Our survey showed the efficacy of HPV vaccine against HPV 16 and 18 infection in Japanese women. The efficacy appear to be different according to the sexual debut age.

WACC II-14

ROMANIAN ADOLESCENTS' KNOWLEDGE AND ATTITUDES TOWARDS HUMAN PAPILLOMAVIRUS INFECTION AND PROPHYLACTIC VACCINATION

C. Maier¹, C.E. Neagu¹, T. Maier¹, R. Vladareanu²

¹University Hospital of Obstetrics and Gynecology "Professor Doctor Panait Sirbu" (Romania), ²Elias University Emergency Hospital (Romania)

Background / Objectives

Since licensure of HPV vaccine in 2006, HPV vaccine coverage among Romanian adolescents remains worryingly low, below 5%(1). The objectives of the study were to assess the knowledge and attitudes towards HPV infection and vaccination among Romanian adolescents and to explore the barriers to HPV vaccination with a view to developing strategies for expanding primary HPV infection prevention.

Methods

This cross-sectional study was conducted in Bucharest between April and June 2015. A total of 524 adolescents aged 16-18 years old were recruited from the first two general highschools in Bucharest (according to the admission grade) and completed a self-administered questionnaire including demographics, HPV related and Papanicolau smear test knowledge. Odds ratio and 95% confidence intervals were used to identify the strength of association. Logistic regression analysis was used to identify the effect of demographic characteristics on the level of knowledge and HPV vaccination rate. Associations were considered statistically significant at $p < 0.05$.

Results

Of the adolescents interviewed, a very small proportion had heard of HPV infection, HPV vaccine and Papanicolau smear test, that is, 20.2%, 13.74% and 22.9%, respectively. The overall vaccination rate for this group was 1.1%. The most common reason for not receiving the HPV vaccine was the lack of information (80.6%) followed by parents' concerns regarding safety (11%), fear of pain (5.5%) and not being sexually active (2.7%). However, 97.7% of the respondents declared interest in receiving more information about HPV. According to demographic characteristics, age at first sexual intercourse over 16 years old, monthly household income over one thousand euros and self-perceived good relationship with family members were statistically associated on a multivariate logistic regression analysis with a high HPV knowledge score and rate of vaccination.

Conclusion

This study shows a low level of knowledge about HPV infection and prophylactic vaccination among Romanian adolescents which may be one of the most important factors for the alarmingly low HPV vaccination rate. We specifically call for HPV knowledge and awareness programs; the implication of health professionals, Romanian media and family members should be included as a centerpiece in the effort to inform this vulnerable population group and to bring the national vaccination coverage rates closer to the ones obtained by other developed European countries.

References

1. <http://www.hpvcentre.net/statistics/reports/ROU.pdf>. Accessed January 31st, 2016.

WACC II-15

HPV VACCINATION INTENTION AMONG MALE CLIENTS OF A LARGE STI OUTPATIENT CLINIC IN AMSTERDAM, THE NETHERLANDS

E. Marra¹, C.J. Alberts¹, G. Zimet², T. Paulussen³, T. Heijman¹, A. Hogewoning¹, G. Sonder¹, H. Fennema¹, H. De Vries⁴, M. Schim Van Der Loeff¹

¹Department of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, The Netherlands (Netherlands), ²Section of Adolescent Medicine, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana, United States of America (United States of America), ³Expertise Center Child Health, Netherlands Organization for Applied Scientific Research (TNO), Leiden, The Netherlands (Netherlands), ⁴Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (Netherlands)

Background / Objectives

In the Netherlands only girls are offered vaccination against human papillomavirus (HPV). Men can become infected with HPV as well, and can develop HPV-induced diseases like genital and oral warts, penile, oropharyngeal and anal cancer. We studied the intention to get vaccinated against HPV, and determinants of intention among male clients of the sexually transmitted infections (STI) clinic in Amsterdam, the Netherlands.

Methods

Men aged ≥ 18 years were recruited from the STI clinic and asked to complete a web-based survey addressing HPV vaccination intention, measured on a 7-point Likert scale (-3 to 3), with the assumption of free vaccination; knowledge about HPV; socio-psychological determinants of HPV vaccination intention; demographics; and sexual behavior. The selected socio-psychological factors were derived from the Theory of Planned Behaviour and Social Cognitive Theory. Univariable and multivariable linear regressions were performed to assess determinants of HPV vaccination intention. Multivariable analysis, using backward elimination, was performed in two steps: univariable associated socio-psychological factors ($P < 0.05$) were entered in the first step, and socio-demographics in the second step. In this second step, all significant variables of the first step remained in the model, regardless of their significance in the second model.

Results

Between June and October 2015, 1490 men participated; 1053 (71%) were men who have sex with men (MSM). The median age was 33 years (IQR 25-44); MSM were significantly older than heterosexual men ($P < 0.001$). The median HPV knowledge score was 5 (IQR: 4-6) of a maximum of 7. HPV vaccination intention was very high (median: 3 [IQR: 2-3]). In multivariable analysis attitude, self-efficacy, descriptive norm, subjective norm, anticipated regret, and beliefs were significantly associated with HPV vaccination intention ($R^2 = 0.70$). Age, sex group and the number of sex partners in the preceding 6 months also appeared to be associated with HPV vaccination intention, but adding these to the model did not substantially increase R^2 ($R^2 = 0.70$).

Conclusion

HPV vaccination intention among male clients of the Amsterdam STI clinic is very high. Most of the variance in HPV vaccination intention among men can be explained by socio-psychological factors. These data suggest that if HPV vaccination for men would be offered at STI clinics for free, uptake would be very high.

WACC II-16

THE EFFECT OF PAYMENT ON THE HPV VACCINATION INTENTION AMONG MALE CLIENTS OF THE STI OUTPATIENT CLINIC IN AMSTERDAM, THE NETHERLANDS

E. Marra¹, C.J. Alberts¹, G. Zimet², T. Paulussen³, T. Heijman¹, A. Hogewoning¹, G. Sonder¹, H. Fennema¹, H. De Vries⁴, M. Schim Van Der Loeff¹

¹Department of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, The Netherlands (Netherlands), ²Section of Adolescent Medicine, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana, United States of America (United States of America), ³Expertise Center Child Health, Netherlands Organization for Applied Scientific Research (TNO), Leiden, The Netherlands (Netherlands), ⁴Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (Netherlands)

Background / Objectives

In the Netherlands only girls are offered vaccination against human papillomavirus (HPV); free of charge. Men can become infected with HPV as well, and can develop HPV-induced diseases like genital and oral warts, penile, oropharyngeal and anal cancer. We studied the effect of out-of-pocket payment on the intention to get vaccinated against HPV among male clients of the sexually transmitted infections (STI) clinic in Amsterdam, the Netherlands.

Methods

Men aged ≥ 18 years were recruited from the STI clinic and asked to complete a web-based survey addressing HPV vaccination intention, measured on a 7-point Likert scale (-3 to 3); knowledge about HPV; socio-psychological determinants of HPV vaccination intention; demographics; and sexual behavior. The selected socio-psychological factors were derived from the Theory of Planned Behaviour and Social Cognitive Theory. Different amounts of out-of-pocket payment (€50;€100;€200;€350) were proposed to different groups of participants, based on the period of participation (the study duration was divided in 4 periods of roughly equal length). Univariable and multivariable linear regressions were performed to assess the impact of out-of-pocket payment on HPV vaccination intention.

Results

Between June and October 2015, 1490 men participated; 1053 (71%) were men who have sex with men (MSM). HPV vaccination intention was very high (mean: 2.2, SD 1.2) if vaccination was free of charge. However, with each step increase in the required out-of-pocket payment for HPV vaccination, mean HPV vaccination intention decreased 0.74 (95%CI: 0.71 - 0.77) scale-point, to a mean value of -0.75 (SD 1.8) when out-of-pocket payment was €350. Also in multivariable analysis, including socio-psychological factors (attitude, self-efficacy, beliefs, social influences, and anticipated regret), and socio-demographics (age, sex group, and lifetime number of sex partner) HPV vaccination intention decreased by 0.74 (95% CI: 0.71 - 0.78) on the 7-point Likert scale, for each increment of price. Higher age was independently associated with higher intention to vaccinate, regardless of out-of-pocket payment.

Conclusion

HPV vaccination intention among male clients of the Amsterdam STI clinic is very high if free of charge. Out-of-pocket payment has a strong negative impact on HPV vaccination intention. These data suggest that if HPV vaccination for men would be offered at STI clinics, uptake would decrease substantially if out-of-payment would be required.

WACC II-17

DEMOGRAPHIC AND SOCIO-ECONOMIC DETERMINANTS OF HPV VACCINE UPTAKE IN SWEDEN

J. Wang¹, P. Sparén¹, E. Netterlid², I. Uhnoo², L. Arnheim-Dahlström¹

¹Department of Medical Epidemiology and Biostatistics, Karolinska Institutet (Sweden), ²Public Health Agency of Sweden (Sweden)

Background / Objectives

HPV vaccination was introduced in Sweden in 2006. Subsidised opportunistic, school-based and catch-up vaccination regimens were successively implemented for different age groups since May 2007. To pursue high vaccination coverage and equal opportunities for prevention of disease, it is key to understand how the uptake of HPV vaccination is distributed among different strata of the population. Therefore we compared demographic and socio-economic factors among parents to their daughter's HPV vaccination status across different vaccination regimens.

Methods

The study cohort comprised 709,427 girls born between May 1990 and March 2003 and ever resident in Sweden between May 1, 2007 and March 18, 2014. HPV vaccination records were retrieved from Prescribed Drug Register and National Vaccination Register. Their biological or foster parents were identified through the National Multi-generation Register, and parents' country of birth was retrieved from Total Population Register. Parents' education level and yearly income was retrieved from the Longitudinal Integration Database for Health Insurance and Labour Market Studies. We used Cox regression models to investigate the associations between parents' country of birth, education, income and their daughter's HPV vaccine uptake, stratified by vaccination regimens.

Results

The vaccination coverage was around 40%, 45% and 81% for subsidised opportunistic, catch-up and school-based vaccination, respectively. In subsidised vaccination lower participation was seen for girls to mothers who were born outside of Nordic countries (HR:0.42, 95%CI:0.42-0.43), had lower education level (HR:0.47, 95%CI:0.46-0.48) and lower income (HR:0.78, 95%CI:0.77-0.79). In catch-up vaccination, associations with mothers' country of birth (HR: 0.62, 95%CI:0.60-0.63), education (HR: 0.73, 95%CI:0.71-0.75) and income (HR: 0.89, 95%CI:0.88-0.91) were significantly reduced and in school-based vaccination, the corresponding associations for mother's country of birth and

education were even less salient (HR:0.79, 95%CI:0.78-0.80; HR:0.90, 95%CI:0.88-0.92, respectively), while income remained at about the same level (HR:0.91, 95%CI:0.90-0.93). The corresponding figures associated with the fathers were similar to the above.

Conclusion

The school-based HPV vaccination achieved a high coverage with little variation in demographic and socio-economic disparities, compared to the catch-up and subsidised opportunistic vaccination. A school-based HPV vaccination is most effective to reach high vaccination coverage throughout the whole population, although further efforts should be put on improving vaccination coverage among girls in immigrated families.

WACC II-18

THE INFLUENCE OF MEDIA COVERAGE OF ADVERSE EVENTS ON YOUNG JAPANESE WOMEN'S THOUGHTS AND ACTIONS REGARDING HPV VACCINATION: RESULTS OF A WEB-BASED SURVEY

Y. Motoki ¹, E. Miyagi ¹, M. Asai-Sato ¹, Y. Suzuki ¹, T. Enomoto ², J. Wark ³, S. Garland ⁴

¹Department of Obstetrics and Gynecology, Yokohama City University Hospital, Yokohama, Kanagawa, Japan (Japan), ²Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan (Japan), ³Bone and Mineral Medicine, Royal Melbourne Hospital, Parkville, Victoria, Australia (Australia), ⁴Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Parkville, Victoria, Australia, Department of Microbiology, The Royal Children's Hospital, Parkville, Victoria, Australia, Infection and Immunity Murdoch Childrens Research Institute, Parkville, Victoria, Australia, Departme (Australia)

Background / Objectives

Objectives: The human papillomavirus (HPV) vaccination program in Japan has been hindered dramatically since June 2013, as a result of media reports on the adverse events following the HPV vaccine. To improve this situation, we used a web-based questionnaire to analyze the influence of the media on Japanese young women's awareness and actions related to the HPV vaccination.

Methods

Methods: From December 2014 to January 2016, advertising banners targeting women in Japan aged 20 to 29 years were placed on Facebook and on a homepage advertising our cervical cancer advocacy activities. Eligible participants were emailed instructions for accessing a secure website where they were able to complete a web-based survey including questions on their awareness of the HPV vaccination and the adverse events associated with it.

Results

Results: Among the 1188 women who expressed an interest in participating, 655 (55.1%) completed the survey. Participants had high awareness of the HPV vaccine (600/655, 91.7%) and of the adverse events associated with it (528/655, 80.6%). However, only 9.6% (63/655) had discussed the necessity

of receiving the HPV vaccination with doctors and/or medical staff members. Of the 20.9% (137/655) who were vaccinated, 59.9% (82/137) had received the vaccination before the suspension of the HPV vaccine promotion by the Ministry of Health, Labour and Welfare in Japan in June 2013; only 7.3% (10/137) were vaccinated after this date. Most participants (80.6%, 528/655) knew about the adverse events associated with the HPV vaccination, mainly from watching television programs (80.9%, 427/528). As reasons why they did not receive the vaccination, unvaccinated participants (79.1%, 518/655) listed adverse events (61.6%), lack of need for the vaccination (60.6%), and having regular screenings (40.7%).

Conclusion

Conclusions: A series of media reports about adverse events associated with the HPV vaccine caused young women in Japan to avoid HPV vaccination. Stakeholders and the academic community in Japan must deliver an evidence-based and easy-to-understand message about the HPV vaccine directly to women.

WACC II-19

Understanding Attitudes to Cervical Cancer Screening Amongst Young Women

T. Muller¹, R. Brown¹, A. Olsen²

¹Research School of Psychology, Australian National University (Australia),

²National Centre for Epidemiology and Population Health, Australian National University (Australia)

Background / Objectives

Cervical cancer risk can be significantly reduced through HPV vaccination and regular cervical screening. However, in Australia, 42% of eligible women did not screen in the 2012-2013 period (Australian Institute of Health and Welfare, 2015). Young women in particular have lower rates of screening, and there is evidence to suggest that young women's screening attendance is declining in developed countries (Lancucki et al., 2010). Despite this, there is a paucity of research addressing barriers and facilitators in young women that may explain this trend. Additionally, no study has explored the needs of women who have never screened.

Methods

Twenty women aged 25 – 35 were interviewed about their experiences of screening, and barriers and facilitators to screening attendance. Thematic analysis was used to compare and contrast interviews, locating common themes.

Results

Barriers and facilitators to screening differed depending on whether women had attended screening before. Women's first screening experience was dominated by emotional barriers, such as anxiety about an unfamiliar procedure and seeking emotional support from the practitioner carrying out the test. These were also barriers for women who had never screened. In contrast, for women who had already screened, the process of screening was normalised, and practical barriers such as lack of time and forgetting when to screen were more common. For women who had screened, it was convenient for them to screen during routine primary health care appointments, especially when health care providers reminded them that they were due to re-screen.

Conclusion

Education campaigns should be promoted differently depending on whether women have been screened previously. This includes education and communication with women by health professionals who are central to actively promoting cervical screening as a component of primary health care. Women who have not screened before require more emotional support, whereas other women rely on health care professionals for practical reminders to screen.

References

Australian Institute of Health and Welfare. (2015). Cervical screening in Australia 2012-2013 (Cancer series no. 93. Cat. no. CAN 91.). Canberra: AIHW. Retrieved from <http://www.aihw.gov.au/publication-detail/?id=10737420251>

Lancucki, L., Fender, M., Koukari, A., Lynge, E., Mai, V., Mancini, E., ... Patnick, J. (2010). A fall-off in cervical screening coverage of younger women in developed countries. *Journal of Medical Screening*, 17(2), 91–96. <http://doi.org/10.1258/jms.2010.010017>

WACC II-20

IMPACT OF INCOME, RACE, AND GEOGRAPHIC LOCATION ON UPTAKE OF HPV VACCINATION IN OHIO

E. Paskett, T. Sokari, D. Fickle, C. Washington, J. Fisher

Ohio State University (United States of America)

Background / Objectives

Ohio, a Midwestern US state, has three-shot HPV vaccination uptake rates of 35% for girls and 23% for boys, and has diverse populations based on race, ethnicity, income and geography, each potentially with different risks of acquiring high-risk HPV and cervical cancer. As part of Ohio efforts to increase HPV vaccine uptake, we conducted a survey of parents representing the major diverse Ohio populations to understand factors associated with HPV vaccination of their children.

Methods

A cross-sectional, anonymous survey was administered to 156 parents at Ohio events. Eligible respondents self-identified as English speaking/writing parents/legal guardians of one or more child aged 11-17. Analysis examined differences in survey responses to questions about HPV vaccine knowledge, attitudes, and uptake by parent race and household income, child gender, and geography (Appalachia, a rural lower socioeconomic area of 32 counties with less access to health care, vs non-Appalachia).

Results

Overall, survey respondents were female (82%), white (61%), college graduates (46%), married (69%), working full time (80%), had incomes over \$60,000 (57%), and had private health insurance (64%). While 83% of parents had heard of the HPV vaccine, white parents were more likely to have both heard of it (OR=4.6) and to report vaccinating their daughters (OR=3.9) and sons (OR=4.5) than African-American parents. Parents with household incomes less than \$60,000 were 1.5 times as likely to report sons being vaccinated and twice as likely to report daughters being vaccinated. Also, 64% of married parents reported not vaccinating sons and 55% reported not vaccinating daughters. Parents who agreed the vaccine was safe and effective were 5 times as likely to report vaccinating. African-American parents cited lack of HPV vaccine awareness as the top reason for not vaccinating sons and daughters (62.5%, 57.2%, respectively), white, non-Appalachian parents cited the vaccine as too new and not knowing enough about it (41.6%, 54.6%, respectively), while white,

Appalachian parents cited no doctor recommendation (46%) for sons and that daughters were too young (29.4%).

Conclusion

This study highlighted disparities in vaccination rates. Reasons for not vaccinating varied by race and geographic location, with white parents concerned about vaccine newness, African-American parents not knowing about the vaccine, and Appalachian parents not getting a doctor recommendation. These results suggest the need for different messages for each population and that multi-level strategies (including parents and health care providers) need to be implemented to increase HPV vaccination rates among Ohio children.

WACC II-21

SURVEY OF CURRENT KNOWLEDGE AND ATTITUDES TOWARD THE HPV VACCINE AND CERVICAL CANCER PREVENTION IN JAPAN

Y. Suzuki, A. Sukegawa, Y. Motoki, M. Asai-Sato, E. Miyagi

Yokohama City University Hospital, Department of Obstetrics and Gynecology (Japan)

Background / Objectives

The Ministry of Health, Labour and Welfare, Japan, began recommending human papillomavirus (HPV) vaccination in 2011, but halted the recommendation in July 2013 because of widespread public suspicion of adverse events associated with the vaccine. This situation could negatively impact efforts for the prevention of cervical cancer (CC). We sought to investigate and clarify attitudes toward CC prevention, including HPV vaccination, among the general population in Japan.

Methods

We conducted our study from October to December 2015 at different events, including a student lecture, open civic lecture, and university festival. We recruited participants aged 16 years or older. Those who gave consent filled out a 20-question, mark-sheet questionnaire aimed at assessing their knowledge and attitude toward prevention of CC, including use of the HPV vaccination. The questionnaire took around 5 minutes to complete. The Research Ethics Committee of Yokohama City University approved the study.

Results

There were a total of 806 participants, though 103 did not fully complete the questionnaire and were subsequently excluded. We analyzed the remaining 703 (185 males, 518 females). Mean age was 35.5 ± 15.3 (range, 16–84) years. Of the respondents, 59% were medical staff or students, while 41% were not involved in medical fields. The average positive (showed awareness) answer rate for the 11 questions about knowledge related to CC prevention, including HPV awareness and HPV vaccination, was 62.6%. Among male respondents the rate was 59.1%, below the 63.3% for female respondents ($p=0.07$). Males who indicated they learned of adverse effects of HPV vaccination via the media were 65.9%, while females were 74.3% ($p=0.014$). However, 57.8% of males indicated they learned of the effectiveness of HPV vaccination via the media; a rate similar to that of females, 60.8%. Of males,

51.4% indicated they would recommend the vaccination to a daughter, similar to the rate for females, 48.8% ($p=0.931$).

Conclusion

Although our results indicated that knowledge of CC prevention is lower among males than females, males seemed interested in the advantages and disadvantages of HPV vaccination. It is thus important to seek to raise awareness about CC prevention not only for females, but also for males. Males' receptive attitudes toward HPV vaccination showed potential for improving the current generally negative public perception of the vaccine in Japan.

WACC II-22

The Impact of Cultural Differences on Cervical Cancer Screening and HPV Vaccination Rates

S.K. Tay

Singapore General Hospital (Singapore)

Background / Objectives

To investigate, among a homogeneous socio-economic group of nurses, (i) the importance of attributes in defining culture and (ii) the impact of different cultural groups on participation rate of cervical cancer screening and HPV vaccination.

Methods

A printed questionnaire, without personal identifier, was used to collect the data. Statistical analysis was performed on SPSS version 22.

Results

Of 2000 copies of questionnaire delivered, 1621 (81.1%) returned the completed questionnaire. Overall, 1347 (83.0%) responders agreed or strongly agreed that the cultural value was important to them. The most important factors determining cultural value were religion (826 (50.9%)) and ethnicity (726 (44.8%)). Based on these findings, cultural traits of the responders were categorized into 11 groupings.

Healthcare utilization by visits to general practitioners, specialists and gynecologists was similar for the 11 cultural groupings. Of 1589 respondents who provided data on sexual behavior, 838 (52.7%) were in a stable sexual relationship, and 637 (40.1%) had never been engaged in sexual activity. The difference in sexual behavior between the cultural groupings was statistically significant ($p < 0.001$). The overall participation rate of regular 3-yearly Pap smear screening rate was 44% and HPV vaccination rate was 9%. There was no discernible difference between the 11 cultural groupings.

Conclusion

In Singapore, self-reported difference in culture among nurses were most prominently attributable to ethnicity and religion. Difference in culture impacted on their sexual behavior but not their participation rate in Pap smear test and HPV vaccination.

WACC II-23

Acceptance of multipurpose human papillomavirus vaccines among providers and mothers of adolescent girls: a mixed-methods study in five countries

N.A. Vielot¹, S.K. Goldberg¹, S.B. Smith¹, G. Zimet², M. Macdonald³, S. Ramos⁴, K. Morgan⁵, C. Kim⁶, K. Richter⁷, M. Peris⁸, K. Whaley⁹, J.S. Smith¹⁰

¹University of North Carolina at Chapel Hill Gillings School of Global Public Health (United States of America), ²Section of Adolescent Medicine, Indiana University School of Medicine, Indianapolis, Indiana (United States of America), ³Center on Genomics, Race, Identity, Difference (GRID), Duke University, Durham, NC, USA (United States of America), ⁴Centro de Estudios de Estado y Sociedad, Buenos Aires, Argentina (Argentina), ⁵Perdana University, Royal College of Surgeons in Ireland School of Medicine, Kuala Lumpur, Malaysia; Royal College of Surgeons in Ireland, Dublin, Ireland (Malaysia), ⁶Department of Obstetrics and Gynecology, The Catholic University of Korea College of Medicine, St. Paul's Hospital, Seoul, Korea (Korea, republic of), ⁷Department of Medical Virology, University of Pretoria and National Health Laboratory Services, Pretoria, South Africa (South Africa), ⁸Unit of Infections and Cancer, Cancer Epidemiology Research Programme, Institut Català d'Oncologia, Barcelona, Spain (Spain), ⁹Mapp Biopharmaceutical, Inc., San Diego, California, USA (United States of America), ¹⁰University of North Carolina at Chapel Hill Gillings School of Global Public Health; UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina, USA (United States of America)

Background / Objectives

Multipurpose vaccines (MPVs) could be formulated based on the existing human papillomavirus (HPV) vaccine to prevent multiple sexually transmitted infections, including herpes simplex virus (HSV-2) and human immunodeficiency virus (HIV). However, little is known about vaccine providers' preferences for MPVs compared to single-purpose HPV vaccine, or the acceptability of MPVs among mothers of adolescent girls.

Methods

A total of 151 adolescent vaccine providers and 118 mothers of adolescent daughters aged 9-14 were recruited from Argentina, Malaysia, South Africa, South Korea, and Spain. Provider preference for single-purpose HPV vaccine or an MPV that prevents HPV+HSV-2; HPV+HIV; or HPV+HSV-2+HIV was

assessed via quantitative survey. Mothers' attitudes towards MPVs were assessed in twenty focus group discussions (FGDs).

Results

Most providers preferred MPVs over single-purpose HPV vaccine; preference for MPVs was highest in South Africa (96.0%) and lowest in Malaysia (60.7%). HPV+HSV-2+HIV was the most preferred formulation (56%-82%) among providers.

Approximately half of all mothers preferred the MPV; support was most pronounced in South Africa and lowest in South Korea. Convenience and trust in the health care system were the most commonly-cited reasons for MPV acceptance, whereas safety and efficacy concerns were the most common barriers; differences emerged by country. Across FGDs, additional safety and efficacy information was requested, particularly from trusted sources such as health care providers (HCPs).

Conclusion

General acceptance of MPVs among adolescent vaccine providers and mothers of adolescent girls supports their development. While most surveyed providers preferred MPVs, further research should identify barriers among providers who did not. MPV acceptance among mothers varied by country, but a common need for safety and efficacy information from HCPs indicates that clinicians are critical to vaccine promotion and acceptance.

SS 01-01

Willingness to "Pay" and the Value of Information for Policy and Research

E. Myers

Duke University School of Medicine (United States of America)

Background / Objectives

Most health decisions involve trade-offs between harms (such as false positives or overdiagnosis) and benefits (such as cancer deaths prevented). Individuals making decisions, or policy makers developing guidelines, are often faced with uncertainty about the magnitude of both harms and benefits for a variety of reasons including wide confidence intervals, biased study designs, and lack of data applicable to a specific setting. In economic analysis, value of information (VOI) is an explicit approach used to estimate the degree to which reducing uncertainty about a decision reduces the chances of making a "wrong" decision, and whether obtaining additional information through research is worthwhile. This approach can be extended to clinical decisions using harm-benefit trade-offs. One of the key insights of this approach is that the chances of making the "wrong" decision, or the "wrong" recommendation, depend on how much one is willing to "pay" for a given benefit. In economic analysis, this is depicted using cost-effectiveness acceptability curves, which show how the likelihood that a given choice is optimal varies as the willingness to pay (for example, in euros per quality-adjusted life year) varies. In a harm-benefit analysis, the curve depicts how the optimal decision changes with the number of harms one is willing to accept in order to gain one benefit.

Methods

Simple models of screening incorporating estimates of sensitivity, specificity, and disease prevalence illustrate how imprecision in estimates of these characteristics affects the harm-benefit ratio in terms of false-positives per true disease detected, and how the optimal test strategy changes as willingness to "pay" for disease detection with false-positives increases. Similar results can be shown with more complex models. By isolating individual components of the decision, the relative reduction in uncertainty by obtaining more precise or less biased estimates can be determined, and used to prioritize additional research.

Conclusion

Arguments about screening recommendations frequently focus on the estimates of harm or benefit, rather than on the inherent trade-off between the two. Using harm-benefit curves has the potential to facilitate evidence-based guidelines by focusing discussion on how the uncertainty in current estimates of harm and benefit affects optimal decisions at different thresholds of acceptable harm. Frank discussions between patients, clinicians, and other stakeholders about what that threshold should be can help make guidelines more transparent, and help identify priorities for future research.

SS 01-02

Using Multi-Scale Models and Value of Information Analysis to Bridge Basic and Population Science and Set Research Priorities

M.D. Ryser

Department of Mathematics, Duke University (United States of America)

Background / Objectives

Quantitative modeling plays an important role in medical decision-making, especially where high-quality data from randomized controlled trials is missing. A key challenge in developing such models is the integration of data and knowledge sources across different physical and temporal scales.

Methods

In this talk, we discuss how mathematical multi-scale models can be used to bridge the gap between biological mechanism and population level data. Based on research examples, we illustrate how these models can be used to evaluate the impact of biologic uncertainty at the decision level, and conversely, how population-level data can be used to improve our understanding of the underlying biologic mechanisms. Finally, we discuss how such models enable targeted prioritization of future research efforts through value of information analyses.

Conclusion

Multi-scale models constitute a valuable bridge between basic science research at the micro-scale and population level data and medical decision-making at the macro scale.

SS 01-03

Going from Research Evidence to Cervical Cancer Control in Populations: The Role of Dissemination and Implementation Research

S. Sivaram¹, C. Vinson²

¹Center for Global Health, US National Cancer Institute (United States of America),

²Division of Cancer Control and Population Sciences, US National Cancer Institute (United States of America)

Background / Objectives

It is estimated that it takes 17 years to translate 14% of evidence from original research to the benefit of patient care. In order to apply scientific data to make decisions, there is need to understand better how to take evidence- often generated in controlled conditions- and bring it to public health settings – also called “real-world” settings - that are often subject to variation and instability. One of the most critical issues impeding improvements in public health today is this enormous gap between our knowledge of strategies that can optimize health promotion and disease prevention and what actually gets implemented. Dissemination and implementation research is a field of inquiry that investigates the process of bringing solutions from biomedical research to public health practice and policy.

The purpose of this talk is to present the principles of dissemination and implementation research as they pertain to cervical cancer control; offer examples of research questions and programs from high-income and low-income countries that seek to bridge this gap between evidence and practice; and discuss research findings. These findings will highlight the role of individual-level, community-level, health systems-level and policy-level factors and discuss how these multi-level factors influence access as well as participation in cancer prevention, early detection, screening, and treatment programs. This talk seeks to promote discussion about dissemination and implementation research and will end with an outline of opportunities for research supported by the US NCI.

Methods

The paper is based on an analysis of the reserach and training programs in dissemination and implementation research both globally and nationally that are supported by the US National Cancer Institute.

Conclusion

Cervical cancer research has generated evidence that offers multiple benefits to populations - it can be prevented with a vaccine; there are tools available to detect it early and manage clinically. Despite this, cervical cancer is responsible for more than 270 000 deaths annually, 85% of which occur in developing countries (World Health Organization). The authors of this paper argue that in order to translate this evidence in cervical cancer research to the benefit women and communities worldwide, the tools of dissemination and implementation research might offer practical prevention and control strategies.

SS 01-05

UTILITY OF VACCINATION, SCREENING, AND CANCER REGISTRIES: HYPOTHESIS GENERATION AND PROGRAM EVALUATION

J. Brotherton

**National HPV Vaccination Program Register, VCS, East Melbourne, Victoria
(Australia)**

Background / Objectives

Objective: To describe the role of vaccination, screening and cancer registries as powerful sources of data to generate hypotheses and evaluate programs.

Methods

In this presentation I will describe the functions, operations and outputs of population based registers supporting vaccination, screening and cancer control programs. I will provide examples of how registers have been utilised to generate hypotheses for further examination and to evaluate programs, including examples relating to HPV vaccination programs and cervical screening programs.

Conclusion

Investing in registries, as part of a comprehensive cancer control program, will provide substantial benefits to support both effective program implementation and to generate useful information to measure program outcomes and effectiveness in the population.

SS 02-01

THE HPV FASTER CONSORTIUM : THE COMBINED VALUE OF HPV SCREENING AND HPV VACCINATION OF WOMEN IN SCREENING AGES

F.X. Bosch José

Catalan Institute of Oncology / IDIBELL (Spain)

Background / Objectives

Session date: 16/June/ 2016

Session number: SS 02

Screening and vaccination interaction: the current perspective

How to best combine vaccination and screening to optimize cervical cancer prevention: the HPV faster consortium

Methods

In spite of the availability of cervical cytology and HPV screening technologies and more recently, of prophylactic HPV vaccines, cervical cancer remains amongst the three most common cancers in women and consistently the second most common in developing countries. In Europe, some 50,000 new cases of cervical cancer occur yearly with a 40 to 60 % mortality rate and great social inequality. Half of these cases occur in the western countries in Europe where screening activities have been in place for decades.

HPV screening has proven to be able to clear prevalent lesions / women at high risk with sensitivities greater than 90% and technology is available that is adaptable to environments with different levels of development. HPV screening is only slowly replacing cytology as the primary screening option.

Current HPV vaccination programs in most countries target single or few cohorts of girls and young women. Phase III clinical trials with vaccines against HPV 16 and 18 have recently shown that protection is also very high for adult women (to ages 45+) provided they are HPV DNA negative at the time of vaccination. A novel HPV vaccine (Gardasil 9) has been licensed including antigens to 9 HPV types (6, 11, 16, 18, 31, 33, 45, 52 and 58) that cover 90% of the infections that cause cervical cancer worldwide.

Conclusion

Extending the age of vaccination to adult women combined with an adequate HPV screening and triage algorithm should be able to dramatically reduce mortality in areas of high risk. These campaign-type approaches have the potential to advance the reduction of cervical cancer incidence and mortality as compared to the time table in the reductions expected if only current programs of vaccination adolescent girls are maintained.

SS 02-03

HPV BASED SCREENING EFFORTS: TRANSLATING DATA INTO IMPLEMENTATION PROGRAMS - THE CASE OF EL SALVADOR

M. Maza¹, K. Alfaro¹, J. Gage², P. Castle³, K. Jane⁴, J. Felix⁵, M. Cremer⁶

¹Basic Health International (El Salvador), ²National Cancer Institute / NIH (United States of America), ³Albert Einstein College of Medicine / Global Coalition Against Cervical Cancer (United States of America), ⁴Harvard T.H. Chan School of Public Health (United States of America), ⁵University of Southern California (United States of America), ⁶Cleveland Clinic / Basic Health International (United States of America)

Background / Objectives

El Salvador has one of the world's highest cervical cancer incidence and mortality rates, at 37.2/100,000 and 18.2/100,000, respectively. In an effort to improve cervical cancer screening in El Salvador, the Ministry of Health, in cooperation with Basic Health International, has included additional screening technologies, such as visual inspection with acetic acid and an HPV-DNA-based screening demonstration project into their public sector health system.

Methods

Consensus amongst stakeholders from the Ministry of Health, the OBGYN Society, International agencies, non-profits, and other public sector institutions that work on cervical cancer prevention was obtained to develop and support the demonstration project. Training of public sector physicians, nurses, health promoters, and laboratory technicians about HPV screening methods and guidelines was conducted. Research about cost-effectiveness, adherence to recommended screening, screening acceptability, and follow-up were conducted.

Results

The Ministry of Health of El Salvador received a donation of careHPV tests as part of a 3-phase implementation project. This project will provide population-based screening for approximately 30,000 women aged 30-59 living in the Paracentral Region of El Salvador. Currently at Phase 3, 20,000 women have been screened through this project, and it is expected to reach 30 thousand women by 2016. Based on the evidence from the implementation project and the new World Health

Organization's Guidelines, national cervical cancer guidelines have been developed by the Ministry of Health. This was done in order to update algorithms, which include HPV testing and management strategies for HPV positive women. The Ministry of Health is expected to continue scaling up screening with HPV testing until national coverage is reached by 2019.

Conclusion

Implementation of an alternative cervical cancer screening approach in a low resource setting may be feasible but it requires advocacy, training, constant monitoring, and evaluation as well as a research component which will lead to evidence-based policy changes.

SS 02-04

SCREENING AND VACCINATION INTERACTION: THE CURRENT PERSPECTIVE: INTEGRATION OF VACCINATION AND SCREENING: AN EPIDEMIOLOGIC PERSPECTIVE FROM AUSTRALIA

S. Garland

1Head of Clinical Microbiology and Infectious Diseases, Royal Women's Hospital, (Australia)

Background / Objectives

Background: Annually, HPV-related cancers represent nearly 10% of all new cancers in females worldwide with ~14% in the developing-world regions. We have safe, immunogenic, efficacious prophylactic HPV vaccines which prevent vaccine-related HPV infection and disease. Yet despite licensure of HPV vaccines in over 130 countries, with 64 countries having such vaccines in their National immunisation programmes, the actual percentage of eligible females vaccinated in the world is less than 2%.

Objectives: In countries where vaccine coverage is high, already there is seen a reduction in vaccine-related HPV genoprevalence, as well as short incubation diseases (genital warts, cervical lesions). Consequently, the positive predictive value (PPV) of cervical cytology screening, when comprehensive and well quality controlled, in finding cervical lesions becomes unacceptably low. Therefore, more sensitive methods, with greater PPVs, such as HPV DNA are being considered for primary screening.

Methods

Methods & Results: Using Australia as an example, integration of screening and vaccination will be examined. Australia has had public health government funded school-based program for HPV vaccination for young girls since 2007 and boys since 2013. There was a catch up program to 26 years of age for females until the end of 2009. With good vaccine acceptance and high coverage, already vaccine-related HPV genoprevalence has declined markedly in vaccine eligible age groups, together with evidence of herd protection and cross protection of phylogenetically related types. This has translated into marked reductions in cervical lesions, as well as genital warts. Consequently, a comprehensive review of the cervical cytology screening program for females 18 to 69 years, every two years has been made under the "Renewal program," with adoption of HPV DNA primary screening as of May 2017 for women commencing at 25 years of age with 5 yearly intervals.

Conclusion

Conclusion: Australia has embraced prophylactic HPV vaccination, with a gender neutral approach. In addition, with the impact of vaccination reducing vaccine-related HPV disease, primary screening is to change shortly to HPV DNA, commencing at 25 years age.

SS 02-06

Barriers and solutions to increase prevention

M. Steben

Clinique A rue McGill - Montreal (Canada)

Prophylactic vaccines and cervical cancer screening are frequently seen as competitive technologies to control cervical cancer. But evidence shows that neither are an optimal preventive option alone. Combining both technologies may increase the potential for optimal reduction of HPV related cancers such as cervical cancers as well as genital warts. Combining may occur at various time of the sexual life of a woman. Most young women will have had access to HPV prophylactic vaccines at school and will need cervical cancer screening at a later age. Women, who could not access prophylactic vaccines when they were in school, could have access later in life, at the time of cervical cancer screening. Communication and access strategies may differ depending on the degree of HPV prevention program available in their countries. We will see examples of various countries implementation of their dual strategies.

SS 03-01

What do we need to know about HPV disease to inform reliable models of vaccination and screening?

J. Berkhof, M. Van Zummeren, P. Snijders, C. Meijer

VUMC (Netherlands)

Background / Objectives

In future screening programmes, the frequency of screening will decrease because of vaccination. Triage algorithms for high-risk HPV positive women are also expected to change; many candidate triage tests are being evaluated including immunohistochemical tests and molecular tests (e.g. DNA methylation, microRNA). To assess the long-term impact of new screening and triage algorithms, mathematical models may need to be updated based on new insights into disease development. Two questions will be addressed: i) are current models ready to inform about long screening intervals for vaccinated cohorts? and ii) how does the discriminating ability of screening tests vary with the time since onset of HPV infection?

Methods

Logistic trait models will be applied to immunohistochemical, DNA methylation, and viral marker data to characterize the development of a precursor lesion.

Conclusion

Mathematical model predictions are iffy when informing about screening intervals beyond five years, because dwell times in the different precancerous states are uncertain. The discriminating ability of immunohistochemical and molecular tests strongly depends on the time since onset infection and early and late markers can be clearly identified. To evaluate the potential of a late marker as a primary or triage test, an update of current mathematical models seems recommendable.

SS 03-02

What do epidemiological studies tell us about HPV disease?

E. Franco

McGill University - Montreal (Canada)

Since 2000, when the first Markov model of the natural history of HPV infection and cervical cancer was published, there has been much progress in the mathematical and statistical modelling of cervical carcinogenesis with a view to project the impact of new technologies in screening and the role of HPV vaccination. Static Markov models were gradually replaced by dynamic models which simulated the risk of acquisition of individual HPV genotypes and their downstream consequences in risk of cervical precancerous lesions and from that to cancer. The role of natural immunity, herd immunity, hypothetical strategies for HPV vaccination, and algorithms for cervical cancer screening could then be overlaid into ever more sophisticated mathematical models. Economical inputs related to costs, utilities, and discounting based on the expected contribution of preventive interventions were part of the models that generated the bulk of cost-effectiveness analyses that have informed policy making in the control of HPV-associated cancers. These mathematical models have to rely on a credible knowledge base of epidemiological data on HPV transmission and cervical carcinogenesis. The author will summarize the state of that knowledge and on how it has come to assist progress in this area.

SS 03-03

WHAT DO WE KNOW AND WHAT DO WE NEED TO KNOW ABOUT REDUCED-DOSE SCHEDULES?

A.R. Kreimer

**Division of Cancer Epidemiology and Genetics, National Cancer Institute,
National Institutes of Health (United States of America)**

Background / Objectives

Prophylactic human papillomavirus (HPV) vaccination with three doses of commercially available vaccines, the regimen currently approved by the FDA, is highly efficacious in preventing targeted carcinogenic HPV infections and related cervical cancer precursors. In some regions of the world, two-dose vaccination schedules for adolescents have started to be recommended, based on immunobridging studies demonstrating immunologic non-inferiority of two doses in that age group, compared with three doses of the vaccine in the adult women in the phase III efficacy trials.

Methods

The majority of women who are at the greatest lifetime risk for cervical cancer are not being vaccinated because cost and logistical considerations for administering multi-dose vaccine programs continue to impede progress in reducing this now-preventable cancer.

Results

The Costa Rica Vaccine Trial (CVT) and the PApilloma TRIal against Cancer In young Adults (PATRICIA Trial), both of which tested the bivalent HPV vaccine, showed similar vaccine efficacy over four years among women who received one, two and three doses of the HPV16/18 vaccine. Stable antibody responses have been observed throughout the seven years of follow-up accrued to date in CVT, suggesting durability of responses. For the quadrivalent HPV, 36-month preliminary analysis of a large, post-licensure trial in India showed similar protection against HPV16/18 cervical infection whether the women received one dose, two doses, or three doses. However, vaccine recipients in these trials were not randomized to receive these fewer doses, and immunogenicity among one-dose recipients was lower than that observed following two- or three-doses. Thus, the level of evidence in support of single-dose HPV vaccination is insufficient to warrant changes in current recommendations for two- or three-dose schedules.

Conclusion

Reduced-dose schedules for prophylactic HPV vaccines will be discussed, as will the need for additional research, such as a direct evaluation of one-dose efficacy of the HPV vaccines.

SS 03-05

WHAT DO WE NEED TO KNOW ABOUT CANCER CONTROL IN LOW AND MIDDLE INCOME SETTINGS?

S. Franceschi

International Agency for Research on Cancer, Lyon (France)

Background / Objectives

Reducing premature mortality from non-communicable diseases (NCDs) by one third by 2030 is among the target of the new UN Sustainable Development Goals (SDG). In 2012, an estimated 52% of all deaths under age 70 were due to NCDs, and one third-to-half (depending on country) of those deaths was caused by cancer. NCD premature mortality declined globally by 15% between 2000 and 2012 but trends for cancer are less favourable than those for cardio-vascular diseases. In addition, declines have been much greater in high-income countries than in the low- and middle-income countries (LMICs) for which NCD data are limited in quantity and quality. Achieving the SDG target for NCDs will require major interventions to deal with ageing populations, urbanization and lifestyle changes, and will require the generalization of universal health coverage. NCD surveillance is a key element of any such plans including the fight against cervical cancer, the topic of my presentation.

Methods

Among the 14 million new cancer cases that occurred worldwide in 2012, around 2.2 million (15%) were attributable to infections, including 530,000 new cervical cancer cases (1). Prevention of infection-related cancers is shifting from cancer control to infection control, e.g., HPV vaccination and the detection of HPV-infected women. In support of this change, the use of infection-transmission models has entered the field of cancer epidemiology. However, models need to be combined with empirical data and surveillance programs. For cervical cancer, essential information includes the incidence/mortality of HPV-associated cancer (through population-based cancer registries), and the presence and coverage of programs of cervical screening and HPV vaccination. If registries and programmes are not present, the potential for piloting geographically restricted cancer registration and interventions (staff, infrastructure, and treatment facilities for cancer and precancer) should be explored. Surveys on HPV prevalence and lifestyle factors relevant to HPV transmission (sexual habits and sexual networks) would be an additional asset to feed transmission models.

Conclusion

HPV vaccination is much less logistically demanding than cervical screening in LMICs. The implementation of new cancer prevention options and better computing tools should encourage the establishment of routine health statistics also in LMICs.

References

[(1) Plummer M, de Martel C, Vignat J, Ferlay J, Franceschi S. Global burden of cancer attributable to infections in 2012 (in preparation).]

SS 03-06

WHAT DO WE NEED TO KNOW ABOUT CANCER CONTROL IN LOW AND MIDDLE INCOME SETTINGS? A MODELLER'S RESPONSE

M. Jit

London School of Hygiene & Tropical Medicine (United kingdom)

Background / Objectives

Almost 90% of cervical cancer deaths occur in low and middle income countries. Uptake of HPV vaccination and cervical screening in these settings has lagged behind high income countries. Such interventions are needed in order to achieve UN Sustainable Development Goals on non-communicable diseases. However, the optimal combination of preventive options in such resource-limited settings needs to be carefully selected.

Methods

Mathematical models of the health and economic impact of HPV vaccination and cervical screening can be used to inform decision making about cervical cancer control. Existing models have highlighted the impact and cost-effectiveness of vaccinating girls prior to sexual debut. However, more complex options such as catch-up vaccination, gender-neutral vaccination and the selection of appropriate algorithms for screening will require models that take into account sexual behaviour, HPV epidemiology and co-morbidities (such as HIV co-infection) in these settings.

Conclusion

Investment is urgently needed in both data collection and mathematical modelling to inform decisions about cervical cancer prevention in low and middle income countries.

SS 03-08

What can models tell us about screening in the post-nonavalent vaccine era?

K. Simms¹, M. Smith², J.B. Lew³, H. Kitchener⁴, P. Castle⁵, K. Canfell²

¹Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia and Prince of Wales Clinical School, The University of New South Wales, Australia (UNSW is a previous affiliation for KS, MS and KC) (Australia), ²Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia, Prince of Wales Clinical School, The University of New South Wales, Australia (UNSW is a previous affiliation for KS, MS and KC) and School of Public Health, University of Sydney, Australia (Australia), ³Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia and Prince of Wales Clinical School, The University of New South Wales, Australia (Australia), ⁴Institute of Cancer Sciences, University of Manchester, UK (United kingdom), ⁵Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY and Global Coalition against Cervical Cancer, Arlington, VA (United States of America)

Background / Objectives

Background: A next generation nonavalent vaccine (HPV9; Gardasil-9) is being introduced in several countries. The lifetime risk of cervical cancer in cohorts offered this vaccine will be substantially reduced but it is not known whether cervical screening in these cohorts will remain cost-effective. Models can be used to characterize the outcomes from progressively less intensive screening strategies and thus inform determination of the optimal number of cervical cancer screening tests in a woman's lifetime for individuals or cohorts offered HPV9.

Objective: To evaluate whether cervical screening will remain cost-effective in cohorts offered nonavalent vaccines and if so, to characterize the optimal number of screening tests.

Methods

We performed a modelled evaluation using a dynamic model of HPV vaccination and cervical screening for four countries – the USA, New Zealand (NZ), Australia and England. For each country, we considered local factors including vaccine uptake rates (USA/NZ uptake ~50%; Australia/England uptake >70%);, attributable fractions of HPV9-included types, demographic factors, costs and indicative willingness-to-pay (WTP) thresholds. The most cost-effective screening strategy was assessed for cohorts offered HPV9.

Results

In the USA, four screens per lifetime was the most cost-effective option, with a 34% probability of being the optimal strategy at WTP US\$50,000/LYS, and 84% probability at US\$100,000/LYS. In New Zealand, five screens was the most cost-effective, with 100% probability of being optimal at NZ\$42,000/LYS. In Australia, two screens was the most cost-effective option, with 62% probability of being optimal at AU\$50,000/LYS. In England, four screens was the most cost-effective option, with 32% probability of being optimal at WTP of GB£20,000/QALY, increasing to 92% probability at GB£30,000/QALY

Conclusion

Some form of cervical screening is still likely to be cost-effective in cohorts of young females offered the nonavalent vaccine, even in countries with high vaccine uptake, but the optimal number of lifetime screens varies by country.

SS 04-02

Modelled evaluation of screen-and-vaccinate strategies in high and low resource settings

K. Simms¹, J. Killen², M. Hall², J.B. Lew³, M. Smith⁴, K. Canfell⁴

¹Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia and Prince of Wales Clinical School, The University of New South Wales, Australia (UNSW is a previous affiliation for KS, MS and KC) (Australia), (Australia), ²Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia (Australia), ³Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia and Prince of Wales Clinical School, The University of New South Wales, Australia (Australia), ⁴Cancer Research Division, Cancer Council NSW, 153 Dowling Street, Woolloomooloo, Sydney, NSW, 2011, Australia, Prince of Wales Clinical School, The University of New South Wales, Australia (UNSW is a previous affiliation for KS, MS and KC) and School of Public Health, University of Sydney, Australia (Australia)

Background / Objectives

Background: ‘Screen-and-vaccinate’ refers to the possibility of integrating cervical screening and vaccination approaches in adult women (aged 25-45 years), and is a subset of a larger group of notional strategies for vaccinating broader population groups known as ‘HPV-FASTER’. With screen-and-vaccinate, women would receive primary HPV screening (potentially with partial genotyping for HPV16/18) and then unvaccinated women could be offered vaccination, with the offer potentially contingent on HPV DNA status as determined via the screening test. Modelling will play an important role in assessing the effectiveness and cost-effectiveness of vaccination in this context.

Objective: To discuss some key considerations in modelling screen-and-vaccinate strategies and to present some initial findings, for a number of high and medium-low income countries.

Methods

We used a comprehensive dynamic modelling platform of HPV vaccination and cervical screening to evaluate the outcomes and cost-effectiveness of screen-and-vaccinate strategies, in relation to (1) no intervention; (2) screening- alone; and (3) vaccination of all women in this age group, irrespective of HPV DNA status.

Conclusion

The risks of acquiring a new HPV infection that then progresses to invasive cervical cancer in a women's lifetime is lower in adult women than in adolescent girls, and therefore the lifetime benefits of vaccination in this group is lower than in pre-adolescents. This implies that the vaccination price for vaccination of adult women would need to be lower in order for vaccination of this group to become cost-effective. Targeted vaccination of HPV-negative women (as determined via a primary HPV screening test) may increase cost-effectiveness somewhat.

SS 04-06

Practical examples: Migrants, Inuit and first nations

M. Steben

Clinique A rue McGill - Montreal (Canada)

Migrant women face difficulties interacting with the healthcare system of their new country. Migrant women often ignore issues of sexual health not because they avoid broaching the issue with healthcare providers but more because they ignore the necessary information about sexual issues including cervical cancer screening. Community programs that include peer education, community access and self-sampling may offer flexibility necessary for high acceptance of cervical cancer screening. Including vaccination in the intervention might be more difficult to explain given the sensitivity of an issue such as protecting themselves against a sexually transmitted disorder that might be perceived as a sign of promiscuity in certain cultures.

The poorer general health and lower average life expectancy of Indigenous peoples has already been documented. IARC has published comparison of cancer incidence rates in Indigenous populations relative to their non-Indigenous counterparts in high-income countries. Given the global increases in cancer incidence over the next decades predicted by IARC, understanding the magnitude and profile of cancer among Indigenous peoples provides necessary evidence in developing and implementing targeted cancer control policies to reduce the burden in these communities worldwide. Combining peer education, community access and self-sampling may offer flexibility necessary for high acceptance of cervical cancer screening. Adding HPV vaccination to screening might also be a sensitive issue in women with stable partners. First nations' and Inuits' chronic distrust of modern medicine and research might mandate efforts to include issues of cultural sensitivity in the program.

SS 04-07

HPV vaccination of women in screening ages in Italy- HPV Faster

F. Carozzi

Institute of cancer prevention (Italy)

Background / Objectives

A trial on effective surveillance and impact of vaccination on screening for cervical cancer was conducted in Italy:

- To evaluate the effectiveness of vaccination in 25-year old women, not previously vaccinated, at the time of their first access to cervical cancer screening
- To evaluate the immune response following vaccination at this age
- To study the dynamics of the infection after vaccination, including the possible change in the frequency of non-vaccine HPV types
- To evaluate cytological abnormalities reductions in vaccinated women
- To understand the impact of vaccination on screening activity

Methods

All the 25-year old women invited to the Florence Cervical Cancer Screening program were asked to be enrolled in a vaccination study; at enrolment, women performed Pap test , HPV test , HPV serology test and received free offer of HPV vaccination (Cervarix®). The presence of high risk (HR) HPVs in cervical samples was evaluated by HC2 and HPV genotyping was Performed by INNO-LiPA Genotyping Extra t. HPV antibody testing was performed on an established and validated HPV serology method based on Luminex technology . HPV type specific seroreactivity was evaluated on all samples collected before and after vaccination (3-year post vaccination)

Results

a strong reduction for any HR-HPV infection was observed in vaccinated women compared to unvaccinate (16% vs 21.4%, $p=0.12$), particularly for HPV16 (3.2% vs 6.7%, $p=0.08$) and HPV31,33,45 (2.1% vs 6.5%, $p=0.02$) For women HR-HPV negative at enrolment, results reveal no infections due to HPV16, HPV18 and HPV31,33,45. A strong antibody response was observed for HPV16 and18 but also for HPV16/18 related types .The compliance to the recall at a new screening round after 3 years was 82.3% for vaccinated women in the present study and 77.5% in not vaccinated women , showing that vaccinated women maintained a high adherence to screening

Conclusion

In conclusion our results show a reduction for HR-HPV infections, cytological abnormalities and colposcopy referral rate in vaccinated women at 25yo and at this age the best strategy seems to vaccinate HR-HPV negative women. Considering the high efficacy of HPV vaccine also for adult women and taking into account that cervical cancer screening program are implementing HPV primary test starting from 30 years old, vaccinating HPV negative women could become part of the screening process. As we know, this topic is still a complex issue and different parties are involved: a cost effectiveness model would be useful to assess the best strategy to integrate screening and vaccination; our data could allow developing this model.

SS 05-02

Epidemiology of anal HPV infection among women

E. Chiao

Baylor College of Medicine (United States of America)

Background / Objectives

Squamous cell cancer of the anus incidence has been increasing over the past several decades, among women and men. Historically women have had a higher incidence of anal cancer than men, and recent studies have shown that the incidence rate for cancers of the anus, anal canal and anorectum in all ages and races of women has more than doubled.

Methods

This talk will summarize recent data on the epidemiology of anal human papillomavirus (HPV) infection, anal dysplasia and anal cancer among women, focusing on studies evaluating the epidemiology of these conditions among immunosuppressed women (including HIV positive women) and women with a history of HPV-related pathology of the lower genital tract. Studies that evaluate the rates of HR -HPV infection, most common types of HR-HPV infection, anal dysplasia by cytology, high-grade anal intraepithelial lesions (AIN 2+) on biopsy, and anal cancer rates of women who have different risk factors for anal HPV infection will be discussed. Studies evaluating the incidence and clearance of anal HPV infection, and the risk factors for anal HPV infection anal dysplasia and anal cancer among women, focusing particularly on the association between cervical and anal HPV type concordance, will also be described.

Conclusion

Women who are immunosuppressed (either because of HIV infection or from iatrogenic immunosuppression), and women with other genital HPV infection have higher rates of anal HPV infection, dysplasia and cancer, ongoing and future research priorities regarding ano-genital cancer prevention and anal cancer screening among women will be highlighted.

SS 05-03

SELF- AND PARTNER-ASSISTED ANAL EXAMS TO DETECT ANAL CANCER TUMORS MAY BE FEASIBLE

A.G. Nyitray¹, J.T. Hicks², L.Y. Hwang¹, M.S. Baraniuk³, M. White⁴, S. Millas⁵, O. Nkechi², X. Zhang², M. Ross⁶, E. Chiao⁷

¹Center for Infectious Diseases, Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas (United States of America), ²Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas (United States of America), ³Coordinating Center for Clinical Trials, Department of Biostatistics, The University of Texas School of Public Health at Houston, Texas (United States of America), ⁴Gordon Crofoot MD PA, Houston, Texas (United States of America), ⁵Department of Surgery, McGovern Medical School, University of Texas Health Science Center at Houston, Texas (United States of America), ⁶University of Minnesota, Program in Human Sexuality, Minneapolis, Minnesota (United States of America), ⁷Department of Internal Medicine, Baylor College of Medicine, Houston (United States of America)

Background / Objectives

Digital ano-rectal exams (DARE) are standard of care for persons with HIV in the US given high anal cancer incidence; however, they are underutilized [1,2]. In order to increase utilization, we set out to determine if it is possible that men having sex with men (MSM) might be able to report accurate findings after performing a self- (or partner-assisted) anal exam (SAE). Earlier detection of tumors should reduce anal cancer morbidity and mortality.

Methods

We conducted a feasibility study among 155 MSM, aged 27-80 years. A clinician skilled in performing DARE used a pelvic model to train participants in anal anatomy, the detection of abnormalities (i.e., hemorrhoids, warts, fissures, and tumors), and performing an SAE. Then, the clinician performed a DARE (without immediately disclosing results), and the man (or couple) was left in private to perform a self-anal exam (or partner-assisted anal exam). Men recorded a normal or abnormal finding, and completed a questionnaire. Percent agreement and Cohen's kappa assessed concordance between clinician's DARE and lay person's SAE results. A colorectal surgeon verified the clinician's results in a subset of men.

Results

Men had a median age of 52 years, 17% were Latino, 48% were African American, and 64% were HIV-positive. Only 21% reported a DARE in the prior year. Over 95% of men classified the health of their anal canal correctly. Couples had almost perfect concordance with the clinician ($\kappa=0.84$) while singles had fair concordance (0.23). Both singles' and couples' κ was statistically better than the null of "no agreement" between clinician and participant ($p<0.001$). Clinicians' DARE observed 7 abnormalities while the men's SAEs noted 5 of these (71%). The 2 men who missed the abnormality had 3mm nodules/masses. The 5 men who correctly reported the abnormality had masses 4 mm or larger or hardened scars. Of 10 men who claimed an abnormality when there was no palpable pathology, the clinician's DARE determined that 5 likely palpated stool. More than half (63%) of men reported never checking their anus for an abnormality; however, after performing an SAE, 96% said they would do it regularly if it was recommended (most opting for every month or every few months). Over 90% of men reported the procedure was acceptable and reported confidence in their ability to detect an abnormality. A majority (60%) said they would prefer to do their own SAE rather than go to a doctor for a DARE.

Conclusion

These results suggest that tumors of $\geq 4\text{mm}$ may be detectable by self-palpation among MSM and are encouraging given literature suggesting a high cure rate for anal cancer tumors $\leq 10\text{mm}$. [3]

References

- [1] New York State Department of Health-AIDS Institute: HIVQUAL-US. (2013). HIVQual-US Annual Data Report.
- [2] Ong, J., Chen, M., Temple-Smith, M., Walker, S., Hoy, J., Woolley, I., Grulich, A., & Fairley, C. (2013). The inside story. Physicians' views on digital ano-rectal examination for anal cancer screening of HIV positive men who have sex with men. *J Med Screen*, 20(4), 188-191. doi: 10.1177/0969141313515463
- [3] Ortholan, C., Ramaioli, A., Peiffert, D., Lusinchi, A., Romestaing, P., Chauveinc, L., Touboul, E., Peignaux, K., Bruna, A., De La Roche, G., Lagrange, J.L., Alzieu, C., & Gerard, J.P. (2005). Anal canal carcinoma: Early-stage tumors $\leq 10\text{ mm}$ (T1 or Tis): Therapeutic options and original pattern of local failure after radiotherapy. *Int J Radiat Oncol Biol Phys*, 62(2), 479-485.

SS 06-01

HPV testing as a primary screen in the era of HPV vaccination. A numbers game

E.L. Franco

McGill University - Montreal (Canada)

Since 2006, when the first human papillomavirus (HPV) vaccine was approved, there has been much progress on the prevention of diseases associated with HPV infection, most notably cervical cancer. A decade earlier, the first validated HPV assay was approved in cervical cancer screening. As the two cervical cancer prevention fronts, i.e., primary via vaccination and secondary via screening, progressed more or less in parallel they have begun to intersect in recent years, as it has become clear that effective deployment of these strategies requires integration of resources and planning [1]. The enormous success of school-based vaccination programs and catch-up vaccination in many Western countries have already had an impact in reducing the prevalence of cervical precancerous lesions associated with the vaccine-targeted HPV genotypes [2]. The first cohorts of girls vaccinated against HPV will enter screening age during the next few years. As this happens, there is increasing acceptance that cervical cancer screening should rely on molecular testing for HPV, although screening algorithms differ regarding the need for cytology cotesting, triage method, ages to begin and exit screening, and testing interval. Can screening begin later in life, be done less frequently, and be stopped earlier among vaccinated women than among those who were not vaccinated? The answers to these questions are dependent on society's tolerance for risk. Vaccination will have an impact on screening test performance and practices, ultimately reducing the benefit of screening as a preventive strategy. With the recent approval of a nine-valent HPV vaccine, maintenance of high vaccination coverage, and a pan-mucosotropic HPV vaccine in the horizon, the time will come in 30-40 years when we will have to decide if cervical cancer screening should be stopped [3,4]. It is imperative that we begin to consider the benchmarks of screening benefits and harms that will inform such a discussion.

References

1. Franco EL, Mahmud SM, Tota J, Ferenczy A, Coutlée F. The expected impact of HPV vaccination on the accuracy of cervical cancer screening: the need for a paradigm change. Arch Med Res. 2009; 40(6):478-85.

2. Drolet M, Bénard É, Boily MC, Ali H, Baandrup L, Bauer H, Beddows S, Brisson J, Brotherton JM, Cummings T, Donovan B, Fairley CK, Flagg EW, Johnson AM, Kahn JA, Kavanagh K, Kjaer SK, Kliewer EV, Lemieux-Mellouki P, Markowitz L, Mboup A, Mesher D, Niccolai L, Oliphant J, Pollock KG, Soldan K, Sonnenberg P, Tabrizi SN, Tanton C, Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis.* 2015; 15(5):565-80.

3. Franco EL, Shinder GA, Tota JE, Isidean SD. Sobering realizations in cancer prevention and screening and their lessons. *Prev Med.* 2015 Jul;76:129-31.

4. El-Zein M, Richardson L, Franco EL. Cervical cancer screening of HPV vaccinated populations: Cytology, molecular testing, both or none. *J Clin Virol.* 2016; 76 Suppl 1:S62-8.

SS 06-03

Host and HPV methylation – adjunctive biomarkers that improve CIN3 diagnosis specificity

A.T. Lorincz, N. Vasiljevic, A.R. Brentnall, C. Reuter, R. Banwait, R. Warman, L. Cadman, J. Austin, J. Cuzick

Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London. (United kingdom)

Background / Objectives

HPV DNA tests have excellent sensitivity for CIN3+ but a drawback of viral screening is modest to poor specificity. Women infected with high risk (hr) HPV need triage before referral to colposcopy to reduce adverse outcomes and high costs. Current approaches to triage are cytology, HPV16/18 genotyping, and p16/ki-67 staining. Our main goal has been to develop and validate a superior DNA methylation biomarker panel to triage hrHPV infected patients to colposcopy.

Methods

710 women were selected from a study of 6,000 women attending routine screening. Colposcopy follow-up of cytologically abnormal women within a year of triage revealed 38 with CIN2+. We measured DNA methylation of human gene EPB41L3 and selected genomic regions of HPV16, HPV18, HPV31 and HPV33. The methylation results were combined into a classifier, called S5. Mann-Whitney P, logistic regression log-likelihoods, and areas under the curve (AUC) were calculated, sensitivity and specificity were compared by McNemar's test.

Results

At the pre-defined cut-off, S5 showed better sensitivity for CIN2+ than HPV16/18 genotyping (74% vs 54%, $P=0.04$) in hrHPV positive women, and similar specificity (65% vs 71%, $P=0.07$). When sensitivity was made equal, S5 had a much higher specificity (91%) than genotyping ($p<0.0001$). The AUC of S5 was 0.78 (0.69-0.88) for CIN2+ and 0.84 (0.72-0.97) for CIN3+ ($p<0.001$).

Conclusion

The novel S5 classifier was developed in two colposcopy populations, Predictors 1 and 2 and validated in a screening sample from Predictors 3. Methylation measurement appears to be a

consistent and valid approach to triage and can be done reflexively on screening specimens. Additional validation studies, including archived specimens from the FOCAL HPV screening trial in British Columbia are underway.

SS 06-04

Risk-based screening and triage: The example of p16/Ki-67 dual stain

N. Wentzensen

**Division of Cancer Epidemiology and Genetics National Cancer Institute -
Bethesda (United States of America)**

The main goal of cervical cancer screening is to prevent cancers, which is achieved by identifying cervical precancers that can be treated to prevent progression to invasive cancer. On the population level, cervical cancer screening needs to identify the small group of women at increased risk of cervical cancer, who need further workup and possibly treatment, while reassuring the majority of women that their cancer risk is very low. Recent guidelines efforts have adopted a risk-based approach to develop guidelines for screening, triage, management and treatment. Different risk estimates are only relevant when they translate to different clinical management. There are four relevant groups of risk of cervical precancer: At the lowest risk, women return for screening after regular screening intervals. In the intermediate risk group (below a colposcopy referral threshold and above a regular screening threshold), additional testing or increased surveillance is required. The next level is the colposcopy referral threshold, and at the highest risk level, immediate treatment may be an option. p16/Ki-67 dual stain cytology has been evaluated as a biomarker for primary screening and for triage of cytology and HPV screening and will be used to illustrate the risk-based screening and triage approach, a paradigm for precision prevention.

SS 06-05

Application of precision medicine to cervical cancer prevention: Will menopausal women become the highest risk population?

P. Sasieni

Queen Mary University of London (United Kingdom)

Background / Objectives

APPLICATION OF PRECISION MEDICINE TO CERVICAL CANCER PREVENTION: WILL MENOPAUSAL WOMEN BECOME THE HIGHEST RISK POPULATION?

Precision medicine in which variability between individuals is taken into account is often seen as the antithesis of screening which is often presented as being an approach applied to whole populations. Cancer screening already distinguishes based on age.

Methods

We argue that (i) the upper age of cervical screening is largely expert-opinion based rather than evidence based; (ii) changing demographics (in particular the widespread uptake of HPV vaccination in many populations) mean that the focus of cervical screening should shift towards older women; and (iii) the age for exiting cervical screening should be tailored to an individual's risk and therefore is an application of precision medicine.

Results

It is generally accepted that the upper age for screening in unscreened women should be higher than the age of last screen in previously well-screened women. Many cervical screening programmes and guidelines have statements about the upper age for screening or when it is appropriate to exit from screening but empirical data to justify these recommendations is minimal. What evidence that does exist is primarily from cytology based screening and it is difficult to know whether one could safely exit screening earlier after an HPV negative test (or a negative co-test) than after a cytology negative test. One thing that is widely agreed however is that if one uses HPV testing in 64 year old women, those who test positive should not simply be excluded from screening. An important clinical question is what future screening/management is appropriate for a woman in her mid-60s who is HPV positive and either cytologically or colposcopically normal.

Conclusion

More research is needed to determine how best to exit women from cervical screening in the age of primary HPV testing.

SS 06-06

Optimal methods for cervical cancer screening among HIV positive women

T. Wilkin

Weill Cornell Medicine (United States of America)

Background / Objectives

Women infected with HIV are living longer due to the widespread use of antiretroviral therapy. Early initiation of ART reduces the risk of cancer and infection-associated cancers in particular. However, HIV-infected women remain at increased risk of cervical cancer, especially those living in resource-constrained settings. HPV testing plays a key role in cervical cancer screening for HIV-uninfected women, but the utility in HIV-infected has remained unclear.

Methods

This presentation will review trial and cohort data of cervical cancer screening testing among HIV-infected women. The role of HPV testing and alternative biomarkers for determining risk of cervical HSIL will be examined. We will review recent changes to cervical cancer screening guidelines for HIV-infected women.

Results

High risk HPV testing, in particular HPV 16, predicts the risk of cervical HSIL in HIV-infected and HIV-uninfected settings. HPV testing can be used in HIV-infected women using similar algorithms as for HIV-uninfected women. HPV-based screen and treat approaches hold promise for HIV-infected women in resource-constrained settings. The types and quantity of HPV DNA may improve the specificity for cervical HSIL in such approaches.

Conclusion

In resource-rich settings, recommendations for cervical cancer screening are now similar for HIV-infected and uninfected women. HPV testing can be used to determine the risk of cervical HSIL. Availability of rapid, near point-of-care HPV testing may allow for dissemination of HPV-based

screen-and-treat approaches for HIV-infected women in resource-constrained settings. New biomarkers hold promise for further improvements of cervical cancer prevention strategies.

SS 07-02

EMA review confirms Art 20 referral data does not support a causal link between the vaccines and development of CRPS or POTS

E. Cochino

European medicines Agency (United kingdom)

Background / Objectives

EMA has conducted a review of the evidence surrounding reports of two syndromes, complex regional pain syndrome (CRPS) and postural orthostatic tachycardia syndrome (POTS) in young women given human papillomavirus (HPV) vaccines. These vaccines are given to protect them from cervical cancer and other HPV-related cancers and pre-cancerous conditions.

Methods

The review included published research, data from clinical trials and reports of suspected side effects from patients and healthcare professionals, as well as data supplied by Member States. In reaching its recommendations, the PRAC also consulted a group of leading experts in the field, and took into account detailed information received from a number of patient groups that also highlighted the impact these syndromes can have on patients and families.

Results

Symptoms of CRPS and POTS may overlap with other conditions, making diagnosis difficult in both the general population and vaccinated individuals. However, available estimates suggest that in the general population around 150 girls and young women per million aged 10 to 19 years may develop CRPS each year, and at least 150 girls and young women per million may develop POTS each year. The review found no evidence that the overall rates of these syndromes in vaccinated girls were different from expected rates in these age groups, even taking into account possible underreporting. The PRAC noted that some symptoms of these syndromes may overlap with chronic fatigue syndrome (CFS, also known as myalgic encephalomyelitis or ME). The results of a large published study showed no link between HPV vaccine and CFS. As many of the reports considered in the review have features of CFS and some patients had diagnoses of both POTS and CFS, these results were considered relevant for the current evaluation.

Conclusion

Taking into account the totality of the available information the PRAC concluded that the evidence does not support that HPV vaccines (Cervarix, Gardasil, Gardasil 9, Silgard) cause CRPS or POTS. The benefits of HPV vaccines continue to outweigh their risks.

The safety of these vaccines should continue to be carefully monitored. This should include follow-up of CRPS or POTS reports to determine relevant clinical characteristics, to identify possible cases of POTS and CRPS based on broad search strategies including outcome details and to compare reporting rates against available information on the known epidemiology of POTS and CRPS.

References

EMA HPV vaccines Article-20 procedure - Assessment report

http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/HPV_vaccines_20/Opinion_provided_by_Committee_for_Medicinal_Products_for_Human_Use/WC500197129.pdf

SS 07-03

Human papillomavirus vaccination and risk of autoimmune diseases: a large cohort study of over 2 million young girls in France

R. Dray-Spira

**French National Agency for Medicines and Health Products Safety (ANSM)
(France)**

Background / Objectives

As part of its missions of medicines and health product safety monitoring, the ANSM (French National Agency for Medicines and Health Products Safety) has carried out a pharmaco-epidemiological study in collaboration with CNAMTS (French National Health Insurance Fund for Salaried Workers) on the safety of HPV vaccination based on analysis of the French healthcare administrative databases. This study constitutes one of the components of the national and international vaccine monitoring strategy, in addition to the European risk management plan and national enhanced pharmacovigilance monitoring. It was specifically designed to investigate a potential link between HPV vaccination and the development of autoimmune disease.

Methods

All girls aged 13 to 16 years between 2008 and 2012, covered by the French general health insurance scheme and without history of HPV vaccination nor AID, were included and followed using French nationwide databases. Fourteen neurological, rheumatological, haematological, gastrointestinal or endocrine AID, were identified from hospital stays, long-term illnesses or marker drugs. Their incidence was compared between girls exposed versus non-exposed to HPV vaccination, using a Cox model adjusted for inclusion year, geographical area, socio-economic indicators, healthcare use level and other immunizations.

Results

A total of 2,252,716 girls were included. During a mean follow-up time of 33 months, 37% received HPV vaccine and 4,096 AID occurred. Exposure to HPV vaccination was not associated with the occurrence of 12 out of the 14 studied AID. HPV vaccination was strongly associated with an increased risk of Guillain-Barré syndrome (GBS): incidence rate of 1.36 among the exposed [19 cases] versus 0.37 per 100 000 PY among the unexposed [21 cases]; adjusted HR: 3.96 [95%CI: 1.82 to 8.61]. This association was robust and particularly marked in the first months following vaccination. Under

the hypothesis of a causal relationship, this would result in 1.8 GBS cases attributable to HPV vaccine per 100,000 girls vaccinated. A weak association between HPV vaccination and incidence of IBD was found (adjusted HR: 1.18 [95% CI: 1.01 to 1.38]), which became non-significant after censoring the first three months following vaccination.

Conclusion

This study provides reassuring results with respect to the risk of AID after HPV vaccination, confirming the results of previous epidemiological studies. An increased risk of GBS after HPV vaccination seems likely; nevertheless, this increased risk, if confirmed, would have a limited public health impact. Results do not support a causal association between HPV vaccine and IBD.

SS 08-02

Vaccinating women in screening ages. A multi-country acceptability study in Europe

F.X. Bosch José

Catalan Institute of Oncology / IDIBELL (Spain)

Background / Objectives

Session date 16/june/2016

Session number SS 02

Session title : Screening and vaccination interaction: the current perspective

Presentation title : How to best combine vaccination and screening to optimize cervical cancer prevention: the HPV faster consortium

Methods

The risk of cervical cancer incidence and mortality in women that test HPV negative and are vaccinated with a broad spectrum vaccine is probably very low. Under these conditions the requirements for further screening of these women would be very limited (i.e. one / twice in a lifetime) necessarily based on HPV tests with different options for triage of HPV positive women and may indeed reach a point in which further screening may not be necessary. The potential of such strategies include the acceleration of the reduction of invasive cervical cancer by several years, the reductions in costs of screening programs and the increased quality of life resulting from a reduction of medical procedures both in diagnostics and therapeutics.

The EU 7TH frame supported a pan European study in which the feasibility and acceptability of one of such strategies is to be tested. 11 countries in Europe with long term experience in HPV research are participants of the effort. The study is starting the field work in February 2016. Specific objectives are to explore how the logistics of HPV screening can be articulated with the logistics of vaccination in each country. How women and health care providers accept the idea of HPV vaccinating adult women and some estimates of compliance and safety.

Conclusion

The European study (CoheaHr) is part of an international consortium that currently includes studies in Mexico, Australia, Canada and projects in preparation in Colombia, Argentina, Brazil and China.

SS 08-03

HPV self-sampling in cervical screening – a randomized study

J. Berkhof, I. Improve Study Group

VUMC (Netherlands)

Background / Objectives

About one out of two cervical cancers are diagnosed in women without a history of screening. High-risk HPV self-sampling (i.e. self-collection of cervico-vaginal specimen) may lower the screening barrier and enhance the effectiveness of the screening programme. The diagnostic accuracy of primary HPV testing on self-collected samples needs to be assessed.

Methods

In a randomized study, women participating in the Dutch cervical cancer screening programme are randomly allocated to either self-collection or collection by a clinician. HPV testing is carried out on the collected samples by the GP5+/6+-PCR method. HPV-positive women are cross-tested using the other HPV collection method. HPV positive women are triaged by cytology on the clinician-collected sample. The enrollment started in January 2015 and will be completed in November 2016. Primary endpoints are the clinical sensitivity and specificity of HPV self-sampling for detection of CIN2 or worse.

Results

3,293 women were enrolled in year 2015 and about 9,500 women will be enrolled in 2016. High-risk HPV positivity rates of sampled collected in 2015 were 7.3% for HPV-self sampling and 8.0% for clinical-based sampling. Of 97 women with a positive HPV test and abnormal cytology, 84 scored positive on the HPV cross test.

Conclusion

These intermediate results are supportive of HPV self-sampling as self-collected and clinician-collected samples had similar proportion of positive HPV results. Reliable estimates of the clinical sensitivity can only be made after completion of enrolment.

References

This presentation is part of the CoheaHr session (SS07)

SS 08-04

HERD EFFECTS IN VACCINATED POPULATIONS

S. Vänskä¹, T. Luostarinen², M. Lehtinen²

¹University of Tampere (Finland), ²Karolinska Institutet (Sweden)

Background / Objectives

HPV vaccines are highly efficacious, and significant reduction of high-risk HPV type prevalence is within reach. By model predictions, herd effects are stronger for HPV18 and other faster clearing types than for slow clearing HPV16. An ancillary study was conducted independently of the initial community-randomized HPV-040 trial (NCT 00534638, sponsored by GSK Biologicals S.A.) to verify model-predicted herd effect gained from gender-neutral vaccination of early adolescents.

Objectives: To compare methods for analysing community-specific changes of HPV prevalence in non-HPV vaccinated population due to the herd effect following different HPV vaccination strategies.

Methods

In 2007-9, 80.272 1992-95 born adolescents were invited in 33 communities and 20.153 girls and 11.662 boys participated (1). In 11 Arm A communities 90% of participants, and in 11 Arm B communities 90% of girls, received HPV-16/18 vaccine. In 11 Arm C communities all received HBV-vaccine. HPV prevalence were determined by using a MGP primer system PCR from 5.473 non-HPV-16/18 vaccinated 18.5 year-old girls in 2010-2014. By model predictions, herd effects appear with a delay so that they are stronger for 1994-95 than for 1992-93 birth cohorts. Thus, the herd effect into non-HPV vaccinated girls were determined from the (probability density function of) community-specific changes in the HPV prevalence from 1992-93 to 1994-95 birth cohorts by study arms, both in frequentist and Bayesian ways. These estimates are conservative as the 1992-93 cohorts were also vaccinated.

Results

The herd effect against HPV16 was weak/absent: In Arm A RR 1994-95 vs 1992-93 was 0.94 (with 95% CI 0.64-1.30) and 1.03 (0.71-1.41) in Arm B. As predicted, the herd effects were stronger against HPV18 [A: 0.64 (0.43-0.92); B: 0.74 (0.49-1.05)], and against combined HPV16/18/31/33/45 [A: 0.77 (0.59-0.95); B: 0.90 (0.69-1.11)]. In Arm C the corresponding RR ranged from 0.98 to 1.07.

Conclusion

Herd effects were evident in the community specific analysis with the gender-neutral vaccination strategy.

References

(1) Lehtinen M, et al. Vaccine 33:1284-1290, 2015

SS 08-05

HPV BASED SCREENING – FROM RESEARCH TO PRACTICE

M. Elfström¹, S. Törnberg², J. Dillner³

¹Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Regional Cancer Center of Stockholm-Gotland (Sweden), ²Regional Cancer Center of Stockholm-Gotland (Sweden), ³Department of Laboratory Medicine, Karolinska Institutet, Stockholm; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Primary HPV-based screening has been examined in randomized trials and more recently in few pilot programs. Current recommendations encourage implementation of HPV-based screening in the context of an organized program. Making the transition from trial evidence to real-life settings requires careful monitoring and evaluation. Using a comparative health effectiveness research approach, HPV-based screening has been rolled-out with women randomly allocated to contemporary comparison groups. This has allowed for evaluating effects, logistics, and acceptability of the new screening test in the context of routine practice.

Methods

An example from the organized screening program of Stockholm County will be used to illustrate the comparative health effectiveness research approach. Women over 30 were randomly allocated 1:1 to cytology- or HPV-based screening. Indicators including participation following invitation to screening, detection rates, and acceptability were monitored.

Conclusion

Preliminary results from primary HPV-based screening implementation in the organized screening program of Stockholm County have shown non-inferior attendance compared to cytology-based screening. The overall number of cytologies performed has decreased in the HPV-arm and similar detection rates for high grade lesions were found in both arms. Questions regarding screening test results have increased. Implementing in a randomized-fashion in the context of an organized, routine program has allowed for monitoring and evaluation in real-life practice.

SS 08-06

CIN2+ RISKS OF INCIDENT AND PREVALENT HPV INFECTIONS BY HPV16/18 GENOTYPING AND REFLEX CYTOLOGY: LONG-TERM RESULTS FROM THE POBASCAM STUDY

N. Veldhuijzen, N. Polman, P. Snijders, C. Meijer, J. Berkhof

VU University Medical Center (Netherlands)

Background / Objectives

To assess CIN2+ risks by HPV16/18 genotyping or reflex cytology in prevalent and incident screen-detected high-risk HPV (hrHPV) infections.

Methods

Data from women participating in the randomized Population Based Screening Study Amsterdam (POBASCAM) were used. Women with a prevalent hrHPV infection at baseline and women with an incident hrHPV infection at the second screen – occurring 4 to 9 years after enrolment – were included in the analysis. The age-range at testing was 34-60 years for both groups. Histology results occurring within 14 years after enrolment were obtained. The cumulative risks of CIN2+ were estimated by Kaplan Meier.

Results

Among 730 women with a prevalent hrHPV infection, 217 CIN2+ cases were detected during follow-up (mean 9.0 years; range 0.02-14 years), resulting in a cumulative risk of CIN2+ of 21.2% after four years and of 29.1% after nine years. Among 490 women with an incident hrHPV infection, 94 CIN2+ cases were detected during follow-up (mean 6.0 years; range 0.04-10.0). The corresponding cumulative risk of CIN2+ was 15.4% after four years and 21.3% after nine years.

Risk-stratification by HPV16/18 genotyping of a prevalent hrHPV infection identified women with a 45.5% cumulative CIN2+ risk after nine years, compared to a 20.3% CIN2+ risk among HPV16/18-negative women. The difference in CIN2+ risks nine years after an HPV16/18-positive and HPV16/18-negative incident infection was less pronounced (24.8 versus 19.6%, respectively). Reflex cytology was a strong CIN2+ risk-stratifier for both prevalent hrHPV infections (abnormal cytology: 62.4% versus normal cytology: 15.7%) and incident hrHPV infections (abnormal cytology: 44.9% versus normal cytology: 14.4%).

Conclusion

We have observed that risk triaging of hrHPV-positive women yields different results for prevalent and incident hrHPV infections. This emphasizes the important role of infection duration when defining screening and triage algorithms.

SS 08-07

HPV-BASED SCREENING – OPTIMAL TRIAGE STRATEGIES FOR HPV-POSITIVE WOMEN

G. Ronco

Centre for cancer prevention (CPO)

Background / Objectives

HPV testing is more sensitive but less specific than cytology. Thus some triage is needed.

Methods

n/a

Results

A pooled analysis of 4 RCTs showed no heterogeneity between studies in reduced invasive cancer incidence with HPV vs. cytology despite 3/4 used this approach and ¼ direct referral of all HPV+ women to colposcopy. Conversely, the biopsy rate was double in the HPV vs. cytology arm in the study that applied direct referral while there was no difference between arms in the 3 RCTs that used this triage approach. A number of biomarkers have been studied as possible triage tests, including genotyping, p16 over-expression (alone or combined with Ki67), methylation of human and viral genes and expression of the E6 viral onco-protein. A systematic review is needed.

Conclusion

Triage entails risk stratification. Women should be referred to colposcopy if the probability of carrying a colposcopy-detectable CIN2+ is sufficiently high (e.g. $\geq 10\%$), otherwise re-invited for new testing at an interval such that the risk of developing new cancer before it is very low. Given the long time needed for progression to invasion this mainly depends on the cross-sectional sensitivity of the triage test for CIN3. Strategies with short term HPV repeat entail high overall referral independently of the triage test. Very sensitive immediate triage (e.g. by combination of tests) can be efficient if it allows repeat after long interval, even if immediate referral is high. At the first screening with HPV long lasting lesions can be present. They have high risk of progression to cancer if missed by triage test(s). Conversely, at subsequent screens with HPV such lesions have plausibly been removed and just those recent - thus at low risk of progression to cancer - are present. In addition, for the same

reason, the probability of carrying a high-grade CIN is much lower in a woman found to be HPV-positive after the first screen than in a woman with an HPV infection detected at the first screen by HPV. Thus triage can be much less aggressive at subsequent than at the first screen by HPV. Similar reasoning is true for vaccinated women.

SS 09-01

Next Generation Sequencing

A. Lorincz¹, C. Reuter¹, K.W. Lau¹, C. Gilham², A. Ahmad¹, J. Peto², J. Cuzick¹, T. Wilhelm³

¹Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London (United kingdom), ²Dept of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London (United kingdom), ³Institute of Food Research, Norwich (United kingdom)

Background / Objectives

Fuller application of existing HPV vaccines and test methods can improve cervical cancer control but barriers remain. New technology and improved interventions are needed, especially to improve test acceptance and specificity. Developments in next generation sequencing (NGS) are explored that may radically transform routine molecular diagnostics in 10 to 15 years.

Methods

Review of published scientific NGS papers and consideration of recent research in the laboratory of the Wolfson NGS team.

Results

NGS is an ideal method for biomarker discovery and validation and eventually will also be used as a routine test. We have validated a DNA methylation classifier S5 with good performance. Work is underway on an expansion of the S5 biomarker panel by reduced representation bisulfite sequencing on 350 samples from the ARTISTIC study by comparing hrHPV+ women who developed incident CIN2+ versus normal hrHPV+ controls. Our team has shown that a machine learning bioinformatics method (MS-SPCA) can accurately (AUC>0.9) predict CIN2+ years in advance from public NGS data. MS-SPCA has already produced a list of more than 100 potential candidate methylation biomarker genes. Interestingly a gene near the top of the MS-SPCA list is EPB41L3, which is also the independently discovered high performing human gene in the S5 classifier. Our main current aim is to use quasi-genome-wide studies to capture the essence of the very best biomarker genes into small to medium panels. We are also conducting exome sequencing on ARTISTIC samples to identify nucleotide and copy number variations (SNV/CNV) that may reveal CIN2+ molecular signatures in otherwise normal women. Early data have revealed more than 100 high impact SNV that were shared between HPV16 positive CIN3+ and paired HPV16 normal samples taken years earlier from

the same women. Corresponding SNV were not found in unmatched normal HPV16 positive women. All women with CIN3+ had changes in HLA-DRB1 several years before the lesions were detected.

Conclusion

NGS is complex and expensive and remains predominantly a research activity. NGS can produce vast amounts of data on samples but improved bioinformatics pipelines are urgently needed. The rapid transformation of NGS data into actionable information could allow clinically meaningful disease risk profiling of individual patients for routine use.

SS 09-02

HPV16 Whole-Genome Sequencing of 2364 Cervical Cancers and Controls in the IARC International Studies

G. Clifford¹, **L. Mirabello**², **M. Schiffman**², **V. Tenet**¹, **N. Wentzensen**²,
M. Cullen³, **J. Boland**³, **X. Zhang**⁴, **S. Franceschi**¹, **K. Yu**², **Q. Yang**⁴,
R. Burk⁵, **M. Yeager**³

¹International Agency for Research on Cancer, Lyon, France (France), ²Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, U.S.A. (United States of America), ³Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, U.S.A.; Cancer Genomics Research Laboratory, Leidos Biomedical Research, Inc., Frederick, U.S.A. (United States of America), ⁴Cancer Genomics Research Laboratory, Leidos Biomedical Research, Inc., Frederick, U.S.A. (United States of America), ⁵Albert Einstein College of Medicine, USA (United States of America)

Background / Objectives

High-risk HPV types, as well as variant lineages of HPV16, differ considerably in carcinogenicity despite close evolutionary relatedness, but methods to assess the genetic basis of such variation have been lacking. We seek to leverage the type of next-generation sequencing technologies and analyses that have been developed for human genomics, to study the viral genetic component of cervical cancer risk.

Methods

Using an Ampliseq and Ion Torrent-based method developed at NCI U.S.A., we are sequencing DNA derived from FFPE, frozen biopsies or cervical cell samples from 2364 HPV16-positive women: 1521 cervical cancer cases, 213 CIN2/3, and 630 controls. Cervical samples have been collected worldwide from 37 diverse populations by the International Agency for Research on Cancer (IARC). Using case-control comparisons, we are evaluating associations between viral genetic variation and cancer risk, including (1) comparison of viral lineages and sublineages, (2) individual SNPs, and (3) gene-level associations. We are also studying human genetic variation and ancestry in relationship to HPV16 genetic variation and cancer risk.

Results

At the time of abstract submission, whole viral genome sequences have been obtained and analyzed for 1,550 HPV16-positive samples (1,044 cervical cancer cases, 118 CIN2/3, and 388 controls). Variation in the HPV16 E1 and URR regions are strongly and significantly associated with cervical cancer risk. Completed findings for 2,364 HPV16-positive women in the IARC biobank will present the final results at the meeting.

Conclusion

Based on preliminary data, the chance of this approach defining viral genetic determinants of cervical cancer risk appears high, and we are planning to apply it to other high-risk HPV types. This technology now permits much larger studies and more complete genotype-phenotype examinations of HPV genomes than previously possible.

SS 09-04

DETECTION OF CERVICAL (PRE)CANCER ON THE BASIS OF CERVICOVAGINAL FLUID: INCLUSION OF SEVERAL BIOMARKERS FOR OPTIMIZATION OF SENSITIVITY AND SPECIFICITY

X. Van Ostade¹, W. Tjalma², E. Coen¹, G. Van Raemdonck¹

¹University of Antwerp (Belgium), ²University of Antwerp, University hospital of Antwerp (Belgium)

Background / Objectives

Current screening methods for cervical cancer have several disadvantages and vaccination programs cover only parts of the population for varying reasons. Furthermore, the vaccines are not effective against all HPV types. Specific and sensitive biomarkers may therefore pave the way for a diagnostic test that may further optimize prevention of cervical cancer.

The CervicoVaginal Fluid (CVF) is composed of secretions originating from organs that are part of the female genital tract, including vagina, cervix, endometrium and ovaries; hence the proteome of this fluid contains a wealth of information concerning the physiological status of all of these organs. Since many studies have proven CVF self-sampling as a good sample collection method for subsequent assays, CVF may very well be suited for the development of a simple bedside assay for triage of suspected cases or screening in remote areas.

Therefore, proteomics studies were undertaken to search for suitable protein biomarkers that could be used in the development of an ELISA-like assay.

Methods

In a differential proteomics study, six CVF samples from healthy and six samples from precancerous women were run over a 2D-LC-MS/MS platform and quantified by spectral counting. ELISA was used to validate the results on more samples. Differentially expressed proteins were then introduced into Ingenuity Pathway Analysis (IPA) in order to find cervical cancer-related pathways.

Results

We identified one protein, alpha-actinin-4 (ACTN4), that was present and absent in all CVF samples originating from precancerous and healthy women, respectively. We also found four proteins that

showed > 3x up- or downregulation in one of the two conditions. ACTN4 followed appearance or clearance of the virus in longitudinal CVF samples. Further testing with ELISA showed that the protein could discriminate between the healthy and (pre)cancerous state with a sensitivity and specificity of both 80%. Furthermore, IPA analysis showed that, unlike CVF from healthy women, CVF from precancerous women contains many interconnected proteins involved in (cervical) cancer from which some could be valuable biomarkers too.

Conclusion

These results show that CVF is a body fluid that may contain several biomarkers for detection or follow-up of cervical precancerous women. With the set of potential CVF biomarkers it may become possible to determine a cervical (pre)cancer biomarker combination with increased discriminative power, compared to ACTN4 alone. Therefore, quantification of several biomarkers from this list by ELISA or mass spectrometry (Multiple Reaction Monitoring, MRM) is currently ongoing in order to optimize sensitivity and specificity of the assay.

References

Van Raemdonck, G., Tjalma, W., Coen, E., Depuydt, C. and Van Ostade, X.. Identification of protein biomarkers for cervical cancer using human cervicovaginal fluid. *PLoS One*. 9:e106488 (2014).

SS 09-05

CERVICAL INTRAEPITHELIAL NEOPLASIA AND SPONTANEOUS PRETERM BIRTH: A GENOME WIDE ASSOCIATION STUDY (GWAS)

I. Kalliala¹, R. Cartwright², A. Mitra¹, A. Rodriguez², T. Piltonen³, L. Morin-Papunen³, P. Bennett¹, M.R. Järvelin², M. Kyrgiou¹

¹Institute of Reproduction and Developmental Biology, Department of Surgery & Cancer, Imperial College London (United kingdom), ²Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London (United kingdom), ³Department of Obstetrics and Gynecology, University Hospital of Oulu. (Finland)

Background / Objectives

A minority of women infected with human papilloma virus (HPV) will develop cervical intraepithelial neoplasia (CIN) or cervical cancer, suggesting the presence of innate factors predisposing to chronic infection or tumor development. Untreated CIN has been associated with spontaneous preterm delivery (PTB), but the causal pathway remains unclear. We conducted a genome-wide association study to identify underlying genetic risk variants which might predispose to CIN/cervical cancer only or to both outcomes.

Methods

Using Finland's nationwide Registers we identified 353 women with CIN or cervical cancer and 1868 controls without a history of any cytological abnormalities from Northern Finland Birth Cohort 1966 (NFBC66). Women were genotyped using Illumina arrays and SNP imputation was done across genome using 1000 genomes phase 1 data. In the first stage we ran genome wide analyses for the dichotomous outcome CIN or cervical cancer. In the second stage we re-ran the analyses for PTB (122 cases with spontaneous PTB of <37 pregnancy weeks and 1813 controls with no history preterm birth) only for the single nucleotide polymorphisms (SNPs) considered at least suggestive for CIN or cervical cancer ($p < 1 \times 10^{-5}$).

Results

We identified ten novel SNPs ($p < 5 \times 10^{-8}$) associated with increased risk of CIN or cervical cancer (Figure 1). Two of the top variants were intronic or upstream variants for three protein-coding genes at the same locus 13q22: PIBF1, BORA and MZT1 — all with roles in pregnancy, mitotic cell division

and/or cancer development. Among the 234 SNPs analysed in the second stage, two remained significant for PTB and were associated with protein coding sites: at SEPT8 (regulator of cytoskeletal organization, associated with cellular polarity and carcinogenesis) and one at CAPN1 (associated with both human carcinogenesis and low birth weight in animal models).

Conclusion

We observed variants significantly associated with CIN or cervical cancer as well as loci suggesting a presence of shared genetic susceptibility to both outcomes in this cohort. The protein-coding genes in the loci identified are suggested to have roles both in aetiology of carcinogenesis and regulation of pregnancy. These results are promising but require external replication for confirmation.

SS 09-06

CONSECUTIVE HPV GENOTYPING OF INVASIVE CERVICAL CANCER IN SWEDEN

C. Lagheden¹, C. Eklund¹, H. Lamin², S. Nordqvist Kleppe¹, M. Elfström¹, K. Sundström¹, P. Sparén³, J. Dillner¹

¹Dept of Laboratory medicine, Karolinska Institutet (Sweden), ²Dept of Laboratory medicine, Karolinska University Hospital, Stockholm (Sweden), ³Dept of Medical Epidemiology and Biostatistics, Karolinska Institutet (Sweden)

Background / Objectives

The Swedish National Cervical Screening Registry evaluates and provides data to optimize cervical cancer prevention in Sweden. All cervical cancers diagnosed in Sweden during 10 years, 2002-2011, were identified. 4272 confirmed cancer cases were subjected to a national audit including HPV genotyping.

Methods

Formalin-fixed paraffin-embedded (FFPE) samples were used for HPV genotyping. In between each case-block, a blank-block was sectioned, as a control for contamination. The sections were extracted with a heating method and HPV genotyped using modified general primer (MGP)-PCR and Luminex. "HPV negative" cases are being sequenced.

Results

For a valid result the blank-block had to be negative in all tests and the case-block positive for beta-globin. 1560 cases were analysed until February 2016. The most common type was HPV 16 (47%), followed by HPV18 (15%), HPV 45 (7%), HPV 31 (3%), HPV 33 (2%), HPV 52 (1%), HPV 6 (1%), HPV 56 (1%), HPV 39 (1%), HPV 70 (1%), HPV 58 (1%), HPV 59 (1%) and HPV 35 (1%). Single infection was detected in 78% of cases.

Conclusion

Systematic HPV genotyping of all cases of invasive cervical cancer in a country is readily doable as part of monitoring the effectiveness of prevention and continued monitoring of the HPV-type specific disease burden.

SS 09-07

THE PHYLOGENETIC TREE OF L1 HPV-16 ISOLATE FROM WEST JAVA INDONESIA SHOWED ASIAN AND AFRICAN VARIANTS

E. Sahiratmadja¹, F. Fadhilah², H. Susanto³

¹Dept. Biochemistry Faculty of Medicine, Universitas Padjadjaran, Bandung – Indonesia (Indonesia), ²Working Group of Oncology Faculty of Medicine, Universitas Padjadjaran, Bandung – Indonesia (Indonesia), ³Dept. Obstetrics and Gynaecology, Dr. Hasan Sadikin General Hospital / Faculty of Medicine, Universitas Padjadjaran, Bandung – Indonesia (Indonesia)

Background / Objectives

Persistent human papilloma viruses (HPV) infection is known to play a significant role in cervical cancer development. Previous study in West Java – Indonesia has showed that high risk HPV genotypes infected the cervical tissue in multiple infection, predominantly by HPV-16.¹ Interestingly, HPV-16 circulated in Indonesia have a typical Javanese mutation.² As Indonesia has various ethnicities, HPV circulated in Indonesia may also derive from various HPV-variants. The aim of the study was to explore the phylogenetic tree of HPV-16 infecting the cervical cancer patients from West-Java, Indonesia.

Methods

Viral DNA was extracted from randomly chosen cervical cancer biopsies archive samples in the period of 2011-2012, and was subjected to genotype determination using the diagnostic linear array genotyping test (Roche). After confirming the HPV-16 genotype infection, L1 gene was amplified using self designed primers and sequenced. Phylogenetic tree was constructed using MEGA program.

Results

Phylogenetic analysis revealed that HPV-16 isolates (n=8) from West Java – Indonesia were in the subgroup of Asia and East Asia variants (n=7). Next to Asian variants, the sequence alignment also showed African variant (n=1).

Conclusion

Although in our study none of the isolates had been infected by HPV-16 from European variant, but other study from Jakarta showed HPV-16 from European variant indicating that Indonesia was a melting point place in the past history. Further study in HPV phylogenetic tree in multiple infection from the same cervical tissue may be of great interest.

References

1. Sahiratmadja E, Tobing MDL, Dewayani BM, Hernowo BS, Susanto H. Multiple human papilloma virus infections predominant in squamous cell cervical carcinoma in Bandung. *Univ Med* 2014;33:58-64
2. de Boer MA, Peters LA, Aziz MF, Siregar B, Cornain S, Vrede MA, Jordanova ES, Kolkman-Uljee S, Fleuren GJ. Human papillomavirus type 16 E6, E7, and L1 variants in cervical cancer in Indonesia, Suriname, and The Netherlands. *Gynecol Oncol*. 2004;94(2):488-94.

SS 10-01

HPV PERSISTENCE AND THE HUMAN MICROBIOME

A.B. Moscicki

University of California, Los Angeles (United States of America)

Background / Objectives

More and more data continues to confirm the importance of the human microbiota in human health and disease. Much of the work has been focused on the gut microbiota including its influence on behavior as well as local and systemic diseases. In contrast, less is understood about the role of the vaginal microbiome (VM) in human health and disease. Overall, the diversity of the VM appears less than that of the gut. In addition, the compartments (i.e. distal and proximal vaginal vs exocervical vs endocervical) have not been well characterized; mostly because of the close proximity of these sites sample collection has been difficult. The VM consist predominantly of Lactobacillus (L) species. However, defining “healthy” microbiota is challenging since community clustering seems to be a moving target with variability through the women’s menstrual cycle as well her reproductive age. L. iners is present in all women including those with “dysbiosis whereas L. Crisptus is mostly seen in “healthy” women. Interestingly the gut does appear to be the reservoir of many of the VM but clearly the vagina is protected from colonization of many of the gut species. Most studies have not revealed much about behavior or environmental influences on the VM which may be due to the fact that most studies are cross-sectional. The few studies examining HPV and the VM have done so in cross-sectional analysis and show a trend toward a relatively low abundance of Lactobacillus spp with increase in anaerobes (such as Prevotella and Leptotrichia). New data has emerged that non-hrHPV is likely a member of the commensal microbiome community and may also have a role in control of hrHPV infections. We have the opportunity to examine the VM over a 20 year span in healthy women from the San Francisco HPV natural history study.

Methods

We will present data on VM, immune biomarkers (e.g. cytokine/chemokines) and HPV persistence and clearance as well as examine effects of smoking and contraceptive use; both considered risk factors for cervical cancer development.

Conclusion

The VM is likely critical in controlling hrHPV infections. Environmental influences may include cigarette smoking of which nicotine can be detected from cervical mucous and high estrogen exposure from oral contraceptives.

SS 10-02

Oropharyngeal cancers and the microbiome

M. Goodman

Cedars Sinai Medical Center (United States of America)

Background / Objectives

Microbes live within a complex ecosystem in the human oral cavity and other body habitats involving well over 1,000 different types of bacteria, archaea, eukarya, fungi, and viruses. While the majority of microorganisms exist within the oral cavity in a symbiotic relationship with the host, perturbations of the host-microbiota interaction may lead to oral and pharyngeal disease, including cancer.

Methods

We are just beginning to understand the degree to which indigenous microbiota and viruses increase the risk of malignancy, including oral and pharyngeal malignancies. New sequencing technologies permit the characterization of complex microbial communities that might be involved in oral health and disease.

Results

Results from recent investigations using 16S rRNA gene tag pyrosequencing suggest that 250-300 bacterial phylotypes inhabit the mouth of healthy individuals. Protection against oral infectious diseases is gained through competition between commensal and pathogenic symbionts for mucosal surfaces. Such competition between species not only dampens the risk of disease, but also reduces the concentration of proinflammatory cytokines elicited during infection. Pathogens and commensals in the oral cavity evade the immune response by forming biofilms that are resistant to host clearance. While several small studies suggest differences in the oral microbiome between oral and pharyngeal cancer patients and healthy controls, specific causal pathogens have yet to be identified.

Conclusion

Recent research provides evidence for an increasing percentage of oropharynx cancers attributable to HPV infection. It is likely that other infectious agents may also contribute to the risk of oral and pharyngeal malignancy.

SS 10-03

Vaginal microbiome: how is this affected by cervical precancer and its treatment?

M. Kyrgiou

Imperial College London (United kingdom)

The link between health and disease, and the human microbiome is a fast-moving area of research, and an appreciation of the variation in microbiome structures amongst individuals is expanding our understanding of the pathophysiology underlying a variety of diseases. *Lactobacillus* spp. was first described by Döderlein 1892 and has since been appreciated as a fundamental for maintaining vaginal health. Whilst the vagina remains one of the simplest bacterial communities, yet over a century later we are still only beginning to realise the intricacies and complexities of this ecosystem. Ravel et al. were the first to classify the vaginal microbiota (VMB) according to structure, using high-throughput sequencing of 16S rRNA gene on next generation sequencing (NGS) platforms and assigned 5 different community state types (CSTs); CST I, II, III and V are dominated by *Lactobacillus crispatus*, *L. gasseri*, *L. iners* and *L. jensenii* respectively, and CST IV conversely, is a heterogeneous group typified by depletion of *Lactobacillus* spp. with presence of strictly anaerobic species such as *Gardnerella*, *Megasphaera*, *Sneathia* and *Prevotella*.

There is evidence that women with HPV are more likely to have decreased prevalence of vaginal *Lactobacillus* spp. with CST III and IV most commonly associated with HPV acquisition and persistence. CST II has also been suggested to result in the fastest clearance of an acute HPV infection.

We recently published the first study to describe the VMB in women with CIN in 169 women showing that increasing severity of CIN is associated with higher VMB diversity, and decreasing abundance of *Lactobacillus* spp. The frequency of CST IV was 2-fold greater in women with LSIL, 3-fold greater in HSIL and 4-fold greater in women with ICC, compared to controls, with a reciprocal decrease in frequency of CST I with increasing disease severity. In addition, women with high-grade CIN had significantly greater levels of *Sneathia sanguinegens*, *Anaerococcus tetradius* and *Peptostreptococcus anaerobius* and lower levels of *Lactobacillus jensenii* compared to those with low-grade CIN. The changes in the VMB after local cervical treatment and the impact that this may have on reproduction and the risk of preterm birth has also been a focus.

SS 10-04

The temporal association between daily cervical HPV detection and microbial community state type shifts

P. Gravitt

Dept of Global Health (United States of America)

Our understanding of the epidemiology of cervical HPV infection is derived from long interval sampled assessment of the presence of viral nucleic acid in cervical swab samples. Studies which employ more frequent sampling of the genital tract have discovered a far more dynamic natural history of HPV, which is more closely associated with microenvironmental influences of sex hormones, the microbiome, and mucosal immune function than sexual behavior. Control of highly prevalent HPV infections (with and without carcinogenic potential) may play a previously underappreciated role in overall lower genital tract health beyond the risk of neoplasia.

SS 10-05

PLACENTAL MICROBIOME- A ROLE FOR HPV?

J. Rautava

Institute of Dentistry, University of Turku (Finland)

Background / Objectives

The presence of microbes in the intrauterine compartment during pregnancy has traditionally been investigated in the context of infectious disease and considered a threat to the developing fetus. However, recently the paradigm of a sterile fetal life has been challenged and it seems that there is a naturally occurring microbiome colonizing the healthy placenta. Our understanding of the role of the indigenous microbiota in human reproduction is in its infancy. Nonetheless, there is a growing body of literature supporting the relevance of placental microbes in the establishment of immune tolerance. According to a study by Aagaard et al. (2014), the placenta microbiome is composed of nonpathogenic commensal bacteria and particularly members of the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla. Based on comparison to data from non-pregnant individuals, the placental bacterial microbial profile is reportedly most similar to the oral microbiome.

Methods

By the end of year 2015, 13 studies have to our knowledge been published regarding human papillomavirus (HPV) infection in the placenta. Eleven of the studies (n=35-339) report the prevalence of HPV DNA in the placenta to be 3-75%. HPV DNA has also been detected in placenta samples obtained transabdominally or by cesarean sections, which reduces the likelihood of contamination from the birth canal. HPV DNA has been localized in syncytiotrophoblasts and trophoblasts have been suggested to be the preferential target for HPV. In a cell culture model, HPV16 has been shown to carry out its complete life cycle in trophoblasts and its early proteins reportedly modify trophoblast growth. HPV infection has been suggested to elicit adverse effects on pregnancy. In line with this notion, HPV16 induced abnormal placental growth resulting pregnancy wastage in mouse embryos.

Conclusion

The composition and interactions of the viral, bacterial and fungal microbiome in the placenta as well as its role in health and disease will be discussed in this lecture.

References

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med* 2014;6(237):237ras65

SS 11-03

ASSAY STANDARDIZATION ISSUES AND CDC'S MULTIPLEX ASSAY FOR SEROLOGY

G. Panicker

Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (United States of America)

Background / Objectives

Measurement of HPV antibodies in unvaccinated individuals has been used as measure of lifetime exposure to HPV. In the absence of commercial assays and immune correlates of protection, laboratories have developed a variety of assays, most commonly based on ELISAs with conformationally intact HPV L1 viral-like particles (VLPs) as antigen. With regulatory approval and implementation of HPV vaccines, serology assays, i.e. immunoequivalency in terms of seroconversion and titer, are now being relied on as endpoints in clinical trials of reduced and altered dosing schedules, as well as of new vaccine formulations and biosimilar vaccines. Thus, there is a need to standardize serology assays to improve comparability of results between assay formats and platforms. The WHO HPV Labnet (2006-2011) had taken the first steps to harmonize HPV serology assays through inter-laboratory comparisons and development and validation of International Standards (IS) for HPV 16 and 18. IS allow titers to be expressed in International Units (IU), greatly facilitating comparison of results. Additional needs for standardization include IS for the additional 7 HPV types targeted by current HPV vaccines, reagents and protocols to validate type-specific VLPs, serum panels for setting negative cut-off values and serum panels for ongoing inter-laboratory comparisons. NCI is sponsoring an international meeting on HPV Serology Standardization in March 2016 to assist in assay harmonization, and their recommendations and action plan will be discussed.

Methods

To address the public health needs for HPV serology, CDC searched for a high-throughput multiplex assay platform for reproducible type-specific detection. We used the Meso Scale Discovery (MSD) electrochemiluminescent detection platform to develop a 9-plex direct VLP IgG ELISA. Samples and standard are tested at three point titrations. Antibody titers are calculated in IU or Arbitrary Units/ml in reference to the standard, using the parallel line method. The assay range of detection is ~1000 fold and precision is $\leq 25\%$. Performance was evaluated by comparison with neutralization and competitive Luminex results. Advantages include reduced capture antigen and sample, low backgrounds, fast read times and a large dynamic range. However, multi-spot printing must be performed by the vendor and requires evaluation of spotting quality. The assay has been used to

estimate population and cohort based seroprevalence as well as vaccine response in special populations.

Conclusion

“The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the funding agency.”

SS 11-04

Serology as an endpoint in vaccine research

J. Dillner

Karolinska Institutet (Sweden)

Background / Objectives

HPV vaccination trials have hitherto used clinical endpoints, such as condyloma, CIN, VIN or AIN, with transient or persistent detectability of HPV DNA being used for exploratory analyses. HPV serology has been used to determine eligibility for per-protocol analyses (which have typically been restricted to subjects who are both HPV DNA-negative and HPV seronegative at baseline).

However, with the large-scale and solid evidence on the efficacy of HPV vaccines for preventing clinical disease, it is debatable if it is ethical to use clinical endpoints for vaccination trials (1). Actually, also using infection (HPV DNA persistence) as endpoint is also debatable. It is clearly demonstrated that the infection causes cancer at multiple sites and that the infection can be prevented by vaccination.

The endpoint that still would be possible to use is immunogenicity (HPV Serology). Serology has already been used to establish immunogenicity also in age groups that have not been targeted by efficacy trials (so-called immunobridging studies) and in trials comparing different doses, target groups and vaccine batches (1). A wider use of the HPV serology endpoint for the future is anticipated, for example in trials of second generation vaccines or in basic research evaluating new vaccines or new administration strategies.

The major caveat is that there is only limited international standardization of HPV serology. International Standards (IS) that define an international unit (IU) of HPV antibodies have, for HPV16 and 18, been established by the WHO and can be ordered from the National Institute for Biological Standards and Controls (NIBSC)(2). Their use is described in the WHO HPV Laboratory Manual, resulting in that all laboratories doing HPV16 or 18 serology are able to report the results in IU.

Sourcing and establishment of IS also for antibodies to other HPV vaccine types would be important and will be pursued.

References

1. Primary endpoints for future prophylactic human papillomavirus vaccine trials: towards infection and immunobridging.

Lowy DR, Herrero R, Hildesheim A; Participants in the IARC/NCI workshop on Primary Endpoints for Prophylactic HPV Vaccine Trials.

Lancet Oncol. 2015 May;16(5):e226-33. doi: 10.1016/S1470-2045(15)70075-6.

2. The first international standard for antibodies to HPV16 .

Ferguson M, Wilkinson DE, Heath A, Matejtschuk P.

Vaccine. 2011 Sep 2;29(38):6520-6. doi: 10.1016/j.vaccine.2011.07.007.

SS 11-05

ROLE OF HPV SEROLOGY IN OROPHARYNGEAL CANCER PREDICTION

T. Waterboer

**Infections and Cancer Epidemiology, Molecular Diagnostics of Oncogenic
Infections Division, German Cancer Research Center (DKFZ) (Germany)**

Background / Objectives

Antibodies to HPV16, particularly the E6 oncoprotein, are strongly associated with HPV16-driven cervical cancer (CaCx), however they are a late event in tumor development (low prevalence in women with CIN3), and about half of women with HPV16-driven invasive CaCx do not seroconvert. In contrast, HPV16 E6 antibodies show >95% sensitivity and specificity for detection of HPV16-driven oropharyngeal cancer (HPV-OPC) in cross-sectional studies, and were shown to precede OPC tumor diagnosis by more than 10 years in prospective cohort studies.

Methods

Antibodies to HPV16 L1, E1, E2, E4, E6, and E7 proteins in serum or plasma samples from two case series collected in Europe and the US, and two prospective studies, the European Prospective Investigation into Cancer and Nutrition (EPIC), and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), were analyzed by multiplex serology. Tumor HPV status was determined by HPV in-situ hybridization (ISH), HPV DNA detection, HPV RNA patterns (E6*I, E1^E4 and E1C), and p16 immunohistochemistry (IHC).

Results

Antibodies to HPV16 E6 were shown to be almost exclusively present in cases with HPV-driven OPC, yielding both sensitivity and specificity exceeding 95%. At the same time, odds ratios for OPC prediction on average >6 years prior to diagnosis were >150. Serial annual samples (median 5 per individual) taken up to 13 years prior to OPC diagnosis showed strong and stable antibody levels.

Conclusion

HPV 16 serological markers, especially antibodies to E6, are strongly associated with OPC more than 10 years prior to clinical tumor diagnosis. To date, the trigger for seroconversion (e.g., infection of

the tonsils or base of tongue, malignant transformation of tonsillar crypt epithelium, yet to be described early OPC lesions) is not understood, and the clinical implications of early HPV-OPC detection are under debate.

SS 11-06

Investigation of anti HPV16L1 antibody levels in dried blood spots in unvaccinated women

R. Bhatia¹, **J. Stewart**², **S. Moncur**², **H. Cubie**³, **C. Busby-Earle**¹, **A. Williams**³, **K. Cuschieri**¹, **S. Howie**³

¹Dr (United kingdom), ²Mrs (United kingdom), ³Prof (United kingdom)

Background / Objectives

Human Papillomavirus (HPV) infections are widespread in the general population. Upon natural HPV infection, seroconversion occurs only in 50-70% of infected women and 4-36% of infected men. With increasing immunisation, it is essential to monitor antibody levels to inform effectiveness of vaccine. Additionally, measuring antibody titres provides an alternative method of assessing HPV infection and immune response as a biomarker for disease.

The objective of this study was to assess the presence and compare the titre of anti HPV16 L1 antibodies in serum, dried blood spots and liquid based cytology (LBC) samples from unvaccinated women.

Methods

A prospective sample collection from unvaccinated women attending the NHS Lothian Colposcopy clinics after a diagnosis of cytological abnormality was performed. Ethical approval was obtained from Scotland A Research Ethics Committee (REF 12/SS/0034). Whole blood and LBC samples were collected from 94 women and blood was spotted in Guthrie spot filter papers. Genotyping was performed using the Optiplex HPV Genotyping Kit (Diamex GmbH). Anti HPV16 IgA and IgG was measured in serum, blood spots and LBC from 94 women. Antibody avidity assay was done using Guanidine Hydrochloride and avidity index (AI) was calculated. Linear regression analysis was performed for correlation between IgA and IgG levels within and between each medium of sample.

Results

Linear regression analysis established a significant correlation between the levels of IgG and IgA HPV 16 anti-L1 antibodies in the serum and in blood spot eluates of the patients. There were also significant correlations between serum and dried blood spot eluates for the levels of IgG and IgA for each individual. There was a strong correlation between the levels of IgG and IgA anti-HPV16L1 antibodies in the LBC samples but there was no correlation with serum levels from the same women

for either IgG or IgA. There was no correlation between the levels of antibodies and biopsy grade for serum, blood spots or LBCs. There was a range in levels of anti L1 specific antibody in the cohort of women assessed but serum from women infected with alpha 9 types of HPV showed higher values of both IgG and IgA compared to those infected with other types. The average AI in the sample set tested was 0.35 (95% CI 0.25- 0.45)

Conclusion

Our study shows that HPV-16 IgA and IgG antibodies detected in unvaccinated women correlate represent current infection type but have a low avidity. Also, blood spots can be successfully used to detect HPV-16 antibodies. This has implications in low resource settings and population wide surveillance studies aimed at investigation of antibody levels.

SS 12-02

LONG-TERM RISK FOR NON-CERVICAL ANOGENITAL CANCER IN WOMEN WITH PREVIOUSLY DIAGNOSED HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA: A DANISH NATIONWIDE COHORT STUDY

**F.L. Sand ¹, C. Munk ¹, S.M. Jensen ², M.F. Svahn ¹, K. Frederiksen ²,
S.K. Kjær ³**

¹Unit of Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen (Denmark), ²Unit of Statistics, Bioinformatics and Registry, Danish Cancer Society Research Center, Copenhagen (Denmark), ³Unit of Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen and Department of Gynecology, Rigshospitalet, University of Copenhagen (Denmark)

Background / Objectives

Persistence of high-risk human papillomavirus (HPV) is essential for the development of cervical cancer and high-grade cervical intraepithelial neoplasia (CIN2/3) and has also been associated with non-cervical anogenital cancers including anal, vulvar and vaginal cancer. Thus, CIN2/3 and anogenital cancers share a common aetiology. Still, there is relatively limited knowledge about the long-term risk for non-cervical anogenital cancer in women with a previous diagnosis of CIN2/3.

Methods

In a nationwide cohort study, we followed nearly 2.8 million women born in 1918–1990 who were recorded as living in Denmark between 1 January 1978 and 31 December 2012. The cohort was linked to multiple nationwide registers to obtain information on cancer diagnoses and confounders. Follow-up started when the women reached 18 years, date of immigration or January 1978, whichever occurred latest, and continued until emigration, death, 31 December 2012 or the date of first diagnosis of anogenital or rectal cancer.

Results

Women with a history of CIN2/3 had higher risks for subsequent anal (HR=3.8; 95% CI:3.2–4.5), vulvar (HR=3.6; 95% CI:3.0–4.3) and vaginal (HR=15.1; 95% CI:11.5–19.9) cancer than women with no such history. After adjustment for smoking and education level, a history of CIN2/3 remained significantly associated with non-cervical anogenital cancer. No excess risk was found for rectal

cancer (HR=1.0; 95% CI:0.9–1.2). Analyses in which time since first CIN2/3 was taken into account showed increased relative risks for anal (HR=4.3; 95% CI:3.0–6.1), vulvar (HR=2.9; 95% CI:1.8–4.6) and vaginal (HR=9.7; 95% CI:4.9–19.4) cancers \geq 25 years after CIN2/3 diagnosis.

Conclusion

Women with a history of CIN2/3 have a long-term increased relative risk for the development of anal, vulvar and vaginal cancer. This finding could inform a decision which may include surveillance for non-cervical anogenital cancers in the follow-up programme offered to women diagnosed with CIN2/3.

SS 12-03

ASSOCIATION OF BACTERIAL VAGINOSIS WITH PERSISTENCE OF FEMALE GENITAL HUMAN PAPILLOMAVIRUS INFECTION - A SIX-YEAR FOLLOW-UP-STUDY

K. Kero¹, J. Rautava², K. Syrjänen³, S. Grenman⁴, S. Syrjänen²

¹MD, Ph.D, Department of Obstetrics and Gynecology, University of Turku, Turku, Finland (Finland), ²Medicity Research Laboratory and Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland (Finland), ³Department of Clinical Research, Biohit LTD, Helsinki (Finland), ⁴Department of Obstetrics and Gynecology, University of Turku, Turku, Finland (Finland)

Background / Objectives

Contradictory data exist on the importance of abnormal vaginal microbiota, especially bacterial vaginosis (BV), on the natural history of human papillomavirus (HPV) infections. This is why we studied the effects of BV and yeast infection on outcomes of HPV infection, as well as the risk factors for the outcomes on asymptomatic women participating in the Finnish Family HPV (FFHPV) Study during a 6-year-follow-up (FU).

Methods

Asymptomatic pregnant women (n=329) were enrolled in third trimester of their index pregnancy. Cervical scrapings were collected for HPV testing at baseline and at 6, 12, 24, 36 mo and six-year-follow-up (FU)-visits. At the same time points also a routine cervical pap smear was taken. HPV genotyping was done with nested PCR and Multimetrix Assay to determine the point prevalence and persistence of the HPV infections. Pap smears were scored for BV and yeast infection. Covariates of the outcomes were analyzed using generalized equation (GEE) and Poisson regression.

Results

Altogether, 76.6% (252/329) of the women tested HPV-positive at least once during the six-year FU. BV was detected in 57.4% (189/329) and yeast infection in 22.9% (73/329) of the women. HPV+ samples presented with significantly more abundant leucocytes in the Pap smear (p=0.023). BV (OR 2.75, 95% CI 1.77-4.27) and yeast infection (mild, moderate, severe) (p=0.007) were strongly linked with HPV positivity. In addition, BV was significantly associated with the HPV persistence (p=0.024;

OR 2.15, 95% CI 1.13-4.08) while no such link was found between yeast infection and HPV persistence.

Conclusion

BV and candida infection were associated with prevalent cervical HPV-infection. In the longitudinal setting, BV was linked with HPV persistence implicating a possible significance as an important co-factor for chronic HPV infections.

SS 12-04

RISK OF HPV INFECTION AND CYTOLOGICAL ABNORMALITIES BY HPV VACCINATION HISTORY IN WOMEN 21-34 YEARS OF AGE

T. Wright¹, V. Parvu², M. Stoler³, C. Cooper², K. Yanson², S. Kodsi²

¹Columbia University (United States of America), ²BD Life Sciences (United States of America), ³University of Virginia (United States of America)

Background / Objectives

The HPV vaccines first became available in 2006 and many countries rapidly introduced school-based vaccination programs targeted at specific age cohorts of young women. In countries with high coverage rates such as Australia, dramatic reductions in vaccine-targeted HPV infections and cytological abnormalities have now been documented in the vaccinated age groups. In contrast, in the U.S. vaccination has been opportunistic, many vaccine recipients have been older and coverage rates have been lower. This suggests that the impact of vaccination may be less in the U.S. than in other countries. Few studies have assessed vaccine impact on the prevalence of HPV genotypes and cytological abnormalities in women in the U.S.

Methods

The BD HPV Onclarity study is an ongoing U.S. study involving 31 collection sites in 18 states. 33,858 women 21-83 years were enrolled between August 2013–June 2015. At enrollment, women had a gynecologic examination including a SurePath cytology specimen that was tested for high-risk HPV genotypes using the BD HPV Onclarity assay that provides genotyping information for HPV 16, 18, 31, 33/58, 35/39/68, 45, 51, 52, 56/59/66. We compared the prevalence of HPV genotypes and cytological abnormalities in women 21-34 years stratified by self-reported HPV vaccination history. A Mantel-Haenszel (MH) analysis was performed to determine association between vaccination status and prevalence, adjusting for age.

Results

The Table shows significant differences between unvaccinated (UV) and vaccinated (V) women in the prevalence of HPV 16, HPV 18, and HPV 31.

	21-	24	25-	29	30-	34	
	UV	V	UV	V	UV	V	MH p-value
Number	2380	1424	4118	1187	4678	366	
HPV+	31.1%	27.0%	22.1%	19.4%	15.3%	11.5%	<0.001
HPV 16+	5.1%	1.1%	3.9%	1.6%	2.4%	1.1%	<0.001
HPV 18+	1.8%	0.2%	1.4%	0.3%	1.1%	0.3%	<0.001
HPV 31+	2.6%	1.5%	2.4%	1.0%	2.1%	1.9%	<0.001
ASCUS/HPV+ or LSIL+	12.9%	10.5%	9.1%	7.2%	6.1%	4.1%	0.001
HSIL+	0.7%	0.1%	1.0%	0.8%	1.0%	0.5%	0.049

Minor differences were observed in the prevalence of other HPV genotypes. Moreover the prevalence of cytologic abnormalities is lower. Although the impact of vaccination is greatest in women 21-24 years significant reductions in the prevalence of specific HPV infections and cytologic abnormalities were observed in women 30-34 years of age. This age cohort could have been no younger than 21-25 years at the time of their vaccination, based on study enrollment date.

Conclusion

A prior history of HPV vaccination is associated with significant reductions in the prevalence of HPV 16, 18, 31 and ASCUS-HPV(+)/LSIL+ and HSIL+ in women 21-34 years of age. The data indicates that vaccination of women in the older range of the recommended U.S. catch-up age group provides considerable benefit.

SS 12-05

HIGH-RISK HPV INFECTION IN CERVICAL, ANAL AND ORAL COMPARTMENTS AMONG YOUNG HIV-NEGATIVE THAI WOMEN

E. Tang¹, T. Pankam², S. Kerr³, N. Teeratakulpisarn², S. Nonenoy², W. Kingkaew², P. Rodbamrung², P. Phanupak², N. Phanupak², J. Palefsky⁴

¹Residency Program in Internal Medicine, Kaiser Permanente San Francisco Medical Center, San Francisco, CA (United States of America), ²Thai Red Cross AIDS Research Centre, Bangkok (Thailand), ³HIV-NAT, Thai Red Cross AIDS Research Centre, Bangkok (Thailand), ⁴Department of Medicine, University of California, San Francisco, San Francisco, CA (United States of America)

Background / Objectives

HPV is associated with cancers of the cervix, anus and oropharynx. Few studies have simultaneously looked at HPV infection in all three anatomical sites in healthy 18-26 year old Thai women. Thai girls and women are not yet routinely vaccinated for HPV. Information on HPV DNA at these sites and seropositivity to HPV 16 and 18 is needed to inform the design of HPV vaccination programs for 18-26 year old women in Thailand.

Methods

We recruited 150 healthy HIV-negative women age 18 to 26 with no more than 5 lifetime partners for an HPV vaccine study in Bangkok, Thailand. Cervical HPV DNA was sought using the Cobas HPV test and Linear Array HPV genotyping test. Anal and oral compartments were tested using the Cobas HPV test followed by Linear Array testing of Cobas-positive specimens. A sexual history, socio-demographic information and serum for a pseudovirion-based neutralization assay (PBNA) to detect antibodies to HPV 16 and 18 were also collected at baseline. Fisher's exact test and logistic regression were used to determine if demographic or other health and sexual risk factors were associated with high-risk HPV (HR-HPV) infection in the anus.

Results

Of the 150 Thai women (median age=23 years, median age of sexual debut=19 years), 41/150 (27.3%) women were positive for cervical HR-HPV DNA and 30/150 (20.0%) were positive for anal HR-HPV DNA. Only one participant had HPV (HPV 53) detected in the oropharynx. Anal HPV 16 and 18

DNA were present in 8/150 (5.3%) and 5/150 (3.3%) participants, respectively, and 12/13 (92.3%) with anal HPV 16 or 18 infection had concordant infection in the cervix. 28/150 (18.7%) and 6/150 (4.0%) women were HPV 16- and HPV 18-seropositive, respectively. In bivariate analysis, anal HR-HPV infection was associated with cervical HR-HPV infection ($p < .001$), increased number of lifetime partners ($p = .007$) and past smoking ($P = .05$), but not reported history of anal sex. In multivariable analysis, only cervical HR-HPV infection was associated with high-anal HR-HPV infection (OR 14.8, 95% CI 5.4-40.5; $P < .001$) after adjusting for number of lifetime partners and past smoking.

Conclusion

Anal HR-HPV infection is common among HIV-negative women in Thailand and these women may be at increased risk of anal cancer in the future. Oral HPV infection was uncommon. There was a high degree of concordance between anal and cervical HPV 16 and 18 infection. Anal HR-HPV infection was associated with cervical HR-HPV infection, but not with history of reported anal sex. Our data suggest that many of these infections may be acquired through spread from the cervix. HPV vaccination may still benefit most 18 to 26 year-old Thai women.

SS 12-06

HPV UNVACCINATED STATUS AND HPV SEXUAL RISK BEHAVIORS ARE COMMON AMONG CANADIAN UNDERGRADUATES

W. Fisher¹, M. Zimmerman², T. Kohut³

¹Departments of Psychology and Obstetrics and Gynaecology, Western University, London, Ontario (Canada), ²Department of Obstetrics and Gynaecology, Western University, London, Ontario (Canada), ³Department of Psychology, Western University, London, Ontario (Canada)

Background / Objectives

Background: Since the introduction of HPV vaccination, research has focused on the potential elevation of sexual risk behaviour among vaccinated individuals, while the sexual risk behaviors and accrued risk of morbidity and mortality of infection in the unvaccinated population has been largely ignored (1-3). This research focuses on the sexual risk behaviors of young adult Canadians who have not been vaccinated against HPV despite a publicly funded national vaccination program for females and authoritative recommendation of vaccination for males.

Methods

Methods: 646 Canadian university undergraduates completed a self-administered survey assessing sexual risk behavior and HPV vaccination status. Sexual risk behaviors were analyzed as a function of gender and HPV vaccination status.

Results

Results: 537 (154 men and 383 women aged 17-23) of 646 participants who met eligibility requirements were analyzed. 48.30% (n = 185) of female and 89.61% (n = 138) of male participants had not been vaccinated. 51.35% (n = 95) of unvaccinated women were coitally experienced with a median of 2 partners (range 1 – 100 partners); 55.07% (n = 76) of unvaccinated men were coitally experienced with a median of 2 partners (range 1 – 40 partners). 49.19% (n = 91) of unvaccinated women reported receptive oral sex experience with a median of 2 partners (range 1 – 23 partners); 22.46% (n = 31) of unvaccinated men reported receptive oral sex experience with a median of 2 partners (range 1 – 20 partners). 6.49% (n = 12) of unvaccinated women reported anal sex experience with a median of 2 partners (range 1 – 10 partners); 2.90% (n = 4) of unvaccinated men reported anal sex experience, with a median of 1 partner (range 1 – 15 partners). HPV vaccination

status was not associated with difference in number of vaginal or anal sex partners, age of sexual debut, or consistency of condom use (all $P > 0.05$). Unvaccinated status was associated with the belief that HPV vaccine is not safe ($P < 0.001$), is less effective than claimed ($P = 0.001$), and is promoted by drug companies to make a profit ($P = 0.002$).

Conclusion

Conclusion: Despite availability of a publicly funded vaccination program for women and recommendation of vaccination for men in Canada, a substantial proportion of Canadian young adults sampled remained unvaccinated. Critically, unvaccinated men and women commonly engaged in sexual risk behaviors for HPV infection and generally engaged in sexual risk behaviors at the same level as their vaccinated counterparts. Findings contribute to an evidence-based case for redoubling efforts to encourage HPV vaccination among young unvaccinated individuals who are commonly at risk of HPV infection.

References

1. Fisher, W.A., (2012). Understanding human papillomavirus vaccine uptake. *Vaccine*, 30S: F149–F156.
2. Fisher, W. A., Kohut, T., Salisbury, C., & Salvadori, M. (2013). Understanding human papillomavirus vaccination intentions: Comparative utility of the Theory of Reasoned Action and the Theory of Planned Behavior in vaccine target age men and women. *Journal of Sexual Medicine*. 10:2455–2464.
3. Zimet, G.D., Rosberger, Z., Fisher, W.A., Perez, S., & Stupiansky, N.W. (2013). Beliefs, behaviors and HPV vaccine: Correcting the myths and the misinformation. *Preventive Medicine*, 57: 414-418.

SS 12-07

LOW PREVALENCE OF GENITAL HUMAN PAPILOMAVIRUS AMONG YOUNG HETEROSEXUAL MALES IN AUSTRALIA: EVIDENCE FOR THE IMPACT OF HERD PROTECTION FROM THE FEMALE VACCINATION PROGRAM

D.A. Machalek¹, E.P. Chow², S.M. Garland¹, R. Wigan³, A.M. Cornall¹, C.K. Fairley², J.M. Kaldor⁴, J.S. Hocking⁵, M.Y. Chen², S.N. Tabrizi¹

¹Department of Microbiology and Infectious Diseases, The Royal Women's Hospital and Murdoch Childrens Research Institute, Melbourne, Victoria (Australia), ²Melbourne Sexual Health Centre, Alfred Health and Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria (Australia), ³Melbourne Sexual Health Centre, Melbourne, Victoria (Australia), ⁴The Kirby Institute for infection and immunity in society, University of New South Wales, Sydney (Australia), ⁵Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Victoria (Australia)

Background / Objectives

Vaccination of adolescent females with the quadrivalent human papillomavirus (qHPV; HPV 6/11/16/18) vaccine commenced in Australia in 2007. Subsequently, a decrease in the prevalence of qHPV genotypes has been observed in, both vaccinated and unvaccinated women aged <25 years. In 2013, the program was expanded to include adolescent males. We evaluated genital HPV prevalence in sexually active 16–35 year old unvaccinated heterosexual males.

Methods

In 2014/15, a total of 596 heterosexual males aged 16–35 years were recruited for HPV testing from sexual health clinics and other community based settings across Australia. Participants provided a self-collected penile swab and completed a questionnaire. Genotyping of HPV was performed using Roche Linear Array. Adjusted prevalence ratios (aPRs) were estimated using binomial log linear regression, for qHPV and HR-HPV excluding HPV16/18, comparing unvaccinated men aged <25 to those ≥25 years. PRs were adjusted for source of recruitment, smoking status, age at first sex, and number of female partners in the previous 12 months. Vaccination status was confirmed against the National HPV Vaccination Program Register (NHVPR).

Results

NHVPR and HPV data were available for 477 (80%) participants (median age 23; IQR 20-27). Most (n=465, 97%) had not received the HPV vaccine. Of these, 72% were recruited from sexual health clinics, 29% were current smokers, 44% were ≤ 16 years of age at first sex, and 70% reported ≥ 2 female sexual partners in the previous 12 months. Prevalence of qHPV types was significantly lower in men aged <25 years compared with men aged ≥ 25 years: 3.4% [95% CI: 1.6–6.2%] vs 14.6% [95% CI: 9.7–20.8%] respectively ($p < 0.001$); aPR 0.28 [95% CI: 0.13–0.58%; $p = 0.001$]. Prevalence of other HR-HPV types excluding 16/18 did not change with increasing age and was 17.4% [95% CI: 13.0–22.4%] among men aged <25 years, and 17.0% [95% CI: 12.0–23.1%] among those ≥ 25 years [$p = 0.925$]; aPR 1.06, [95% CI: 0.70–1.60; $p = 0.777$]. Men in the younger age group were significantly less likely to report current genital warts (7.1% [95% CI: 4.3–11.0%] versus 14.7% [95% CI: 9.6–21.3%] among those <25 and ≥ 25 years, respectively [$p = 0.013$]; aPR 0.18, [95% CI: 0.06–0.53; $p = 0.002$]).

Conclusion

In a cohort of highly sexually active unvaccinated heterosexual males we observed a significantly lower prevalence of qHPV types, but not other HR-HPV types, in males aged <25 years. Our results suggest that males may benefit almost to the same extent as females from a female-only vaccination program under high vaccine coverage ($\sim 70\%$), due to herd protection.

SS 12-08

COUNTRY SPECIFIC HPV-RELATED GENITAL LESIONS AMONG MEN RESIDING IN BRAZIL, MEXICO, AND THE UNITED STATES: HIM STUDY

S. Sudenga¹, B.N. Torres¹, W. Fulp¹, R. Silva², L. Villa³, E. Lazcano-Ponce⁴, D. Ingles¹, J. Messina¹, M. Abrahamsen¹, M.L. Baggio³, J. Salmeron⁴, M. Quiterio⁴, A. Giuliano¹

¹Moffitt Cancer Center (United States of America), ²Centro de Referencia de Treinamento em DST e Aids (Brazil), ³Universidade de São Paulo and Instituto do Cancer do Estado de São Paulo (Brazil), ⁴Instituto Nacional de Salud Pública (Mexico)

Background / Objectives

Condyloma (genital warts) is a common outcome of HPV infection and has a high likelihood of reoccurrence. Therefore, treatment is associated with high medical expenditures. Thus, determining condyloma incidence, the HPV genotypes detected in condyloma, and the rate of HPV progression to disease is clinically important, and contributes significantly to cost-effectiveness modeling of prevention and treatment interventions in each country. The purpose of this study was to assess whether the incidence of histopathologically confirmed condyloma and penile intraepithelial neoplasia (PeIN) and rates of genital HPV infection progression to these lesions differs by country among men residing in Brazil, Mexico, and the U.S.

Methods

HPV Infection in Men (HIM) Study participants were men aged 18-70 years living in Tampa, Florida (U.S.), Cuernavaca (Mexico), and Sao Paulo (Brazil). At each visit, condyloma and PeIN lesions were biopsied. Biopsy specimens were categorized by pathologic diagnoses. The Linear Array genotyping method was used to identify HPV genotypes from genital swabs, while the INNO-LiPA HPV Genotyping Extra method was used for biopsy tissue specimens. Age-specific analyses were conducted for lesion incidence by country, with Kaplan–Meier estimation of cumulative incidence by country. The proportion of HPV infections that progressed to condyloma and PeIN, the median time to condyloma and PeIN development, and the incidence rates were estimated by country.

Results

When comparing demographic and sexual characteristics across the three countries, sexual orientation ($p=0.008$) and lifetime number of female sexual partners ($p<0.0001$) were differentially associated with lesion incidence in the three countries. Condyloma incidence rates overall by country were 1.6 per 100 person-years (p-y) in Brazil, 1.8 per 100 p-y in Mexico, and 1.9 per 100 p-y in U.S. Condyloma incidence in Brazil and the U.S. decreased with age, while incidence remained fairly constant across the lifespan in Mexico. No differences by country were observed when comparing PeIN incidence rates across age categories. HPV types 6 and 11 were the most common types to progress to condyloma, and HPV types 16, 6, and 11 were the most common types to progress to PeIN in all three countries.

Conclusion

The continuous risk of condyloma and PeIN across all age groups and countries in this study emphasizes the need to ensure that strong HPV immunity, such as that obtained through vaccination, is maintained across the lifespan of males.

SS 12-09

HPV CLEARANCE AND PERSISTENCY IN YOUNG WOMEN – FIVE YEARS FOLLOW UP OF WOLVES-STUDY

A. Luyten¹, T. Schulz¹, S. Strehlke¹, T. Iftner², A. Iftner², K.U. Petry²

¹Department for Gynecology, Obstetrics and Gynecologic oncology, Wolfsburg (Germany), ²Dept. of Med. Virology and Epidemiol. of Viral Diseases, Tuebingen (Germany)

Background / Objectives

Long term persistency of HPV-HR infections is an essential step in the genesis of cervical cancer. Persistency seems to be rare in young women but the type specific clinical course of HR-infections in young women is still not fully understood.

Methods

WOLVES (Wolfsburg HPV epidemiological study) invited all women born 1988/89 with a first residency in Wolfsburg to participate. Participants were followed with annual examinations from 2009/10 till 2014/15.

Results

Between Oct 2009 and Mar 2014, 990 women were recruited, the HC2 prevalence was 308/1181 (26.1%). Among HC2- women the rate of new infections (HC2+) was 15.7 % (98/623) in the first, 13.4% (52/389) in the second, 10.0% (22/219) in the third and 8.4% (10/119) in the fourth year of follow-up. Overall HPV persistency occurred in 17.3% (148/857) in the first, 13.2% (84/638) in the second, 9.5% (42/441) in the third and 7.5% (19/253) in the fourth year of follow up.

In women with initially negative HPV result (n=848) 182 were tested HPV positive once in 5 year follow up with biopsy proven CIN2+ (0.36%). In HPV positive women (n=308) the risk for CIN2+ into 5 years was 11.7% (n=36). In infections that persisted for one or more follow up visits (n=149), HPV 16 (n=37) / HPV 51 (n=20) / HPV 31 (13) showed the highest numbers and resembled the type distribution among CIN2 cases, while all CIN3 cases were linked to HPV 16.

Conclusion

Spontaneous clearance was lower than reported by others, while HC2- and type specific persistency were more common than expected. Only less than one in two prevalent HPV infections was cleared within two years. We conclude that the risk of HPV persistency in young women may be higher than assumed.

SS 13-01

GENITAL WARTS AND HPV DETECTION IN CHILDREN

A.B. Moscicki

University of California, Los Angeles (United States of America)

Background / Objectives

Although the HPV-associated morbidities in children are less common than seen in adults, several morbidities are seen including recurrent laryngeal papillomatosis (RLP) and genital warts (GW). Perinatal transmission has been thought the primary mode of transmission for RLP and GW.

Methods

The literature was reviewed for HPV DNA detection and disease in children.

Rates of estimated transmission varied widely. The prevalence of oral HPV in neonate ranged from 1%-85%, however, the majority of studies show little correlation between the infant's oral HPV type with the mother's genital type. A recent meta-analysis showed that if the mother had genital HPV, the infant had a 33% increased chance of having oral HPV DNA detected. One study of parents and their neonate observed over a 3 year period a 42% cumulative detection rate of HPV DNA in oral samples and 36% in genital samples. These findings suggest that transmission may also occur between the parent and the infant during caretaking. In a relatively large study, there was a bimodal distribution for oral HPV with 2.5% of those <1 year having a positive test for HPV, 0.15% in the 1-4 year old children and 3.9% of 16-20 year olds. As in adults, source of sample is important in that tonsillar tissue is much more likely to yield a positive HPV test than buccal samples. Studies of foreskin showed higher rates than other anogenital sites. HIV infection as seen in adults may also be a risk for children but little is known about HPV in perinatally infected children (PHIV). A recent study of non-sexually active PHIV found oral HPV in 17% of girls and 4% of boys and genital HPV in 36% and 22%, respectively. GW and HPV DNA detection in children may also be a sign of sexual abuse. Older age of the child at diagnosis is highly correlated with increased risk of sexual abuse. One study showed that HPV DNA was detected from the genital area in 14-16% of those with proven sexual abuse vs 1.3% with no evidence of abuse. In this study 100% of those referred for GW were found to have evidence of sexual abuse.

Conclusion

Several organizations consider GW as evidence of possible sexual abuse if the child is older than 24 months of age when referral for evaluation is warranted. HPV DNA testing is not currently recommended in evaluation. However, organizations now recommend HPV vaccination at 9 years of age in those with a history of sexual abuse. Reasons include that children with a history of abuse are more likely to engage in consensual sexual activity at earlier ages and engage in high risk sexual and substance use behaviors. Early exposure to HPV increases their risk of developing anogenital cancers.

SS 13-02

PREVALENCE OF HUMAN PAPILLOMAVIRUS IN SQUAMOUS VULVAR CANCER AND VULVAR INTRAEPITHELIAL NEOPLASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

M.T. Faber¹, F.L. Sand¹, V. Albieri², B. Norrild³, J. Junge⁴, S.K. Kjær⁵, F. Verdoodt¹

¹Danish Cancer Society Research Center, Virus, Lifestyle and Genes, Copenhagen (Denmark), ²Danish Cancer Society Research Center, Statistics, Bioinformatics and Registry, (Denmark), ³Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen (Denmark), ⁴Department of Pathology, Hvidovre Hospital, University of Copenhagen, Copenhagen (Denmark), ⁵Danish Cancer Society Research Center, Virus, Lifestyle and Genes, Copenhagen and Department of Gynecology, Rigshospitalet, University of Copenhagen, Copenhagen (Denmark)

Background / Objectives

A causal role of human papillomavirus (HPV) has been suggested for a subset of vulvar carcinomas and for the majority of vulvar intraepithelial neoplasia (VIN). A number of studies have investigated HPV prevalence and HPV type distribution in vulvar cancer and VIN, and the findings have been summarized in review articles. Since publication of these reviews, a considerable number of new studies on the association between HPV and vulvar lesions have been published. We conducted an updated systematic review and meta-analysis to estimate the pooled prevalence of HPV DNA and HPV type distribution in vulvar cancer and VIN.

Methods

Through a systematic literature search, studies published between 1990 and 2015 were identified in PubMed and Embase databases, and the Cochrane Library. Studies using a PCR-based or hybrid capture test to evaluate the presence of HPV DNA in invasive squamous cell carcinoma of the vulva or VIN were eligible for inclusion. Pooled estimates of the HPV prevalence with corresponding 95% confidence intervals (CI) were calculated based on a random effects model.

Results

We identified 93 eligible papers examining the prevalence of HPV DNA in vulvar cancer (65 papers) and/or VIN (48 papers) comprising altogether 5106 cases of vulvar cancer and 2415 cases of VIN. The pooled prevalence of HPV in vulvar cancer was 40.1% (95% CI: 35.8–44.7), while the pooled HPV prevalence in VIN was 77.0% (95% CI: 70.7–82.3). Among HPV-positive cases the predominant HPV type was HPV16 (70.9% in vulvar cancer; 81.4% in VIN), followed by HPV33 (13.5% in vulvar cancer; 12.7% in VIN) and HPV18 (10.9% in vulvar cancer; 3.5% in VIN).

Conclusion

HPV prevalence was higher in VIN than in vulvar cancer and HPV16 accounted for the majority of HPV-positive cases. In subsequent meta-regression and stratified analyses, we will evaluate potential sources of between-study heterogeneity such as geographical region, publication year, histological classification, HPV DNA detection method and tissue type.

SS 13-03

RISK FACTORS FOR HIGH GRADE ANAL INTRAEPITHELIAL NEOPLASIA IN WOMEN EVALUATED BY HIGH RESOLUTION ANOSCOPY

C. Bouchard¹, M. Fortier¹, G. Bouchard-Roy¹, A. Bouchard-Fortier²

¹Université Laval (Canada), ²University of Calgary (Canada)

Background / Objectives

High-grade anal intraepithelial neoplasia (HGAIN) is a presumed precursor of anal squamous cell carcinoma (SCC). Risk factors for the development of HGAIN are less clear. Therefore, the aim of this study was to identify risk factors for HGAIN in women undergoing high-resolution anoscopy (HRA).

Methods

Women referred for evaluation of perianal HPV infection, abnormal anal cytology or high risk HPV DNA testing that underwent HRA at our institution were identified from 2013 to 2015. Patient demographics, presence of immunocompromised state (HIV, autoimmune disorder, other malignancy), sexual history (coitarche, number of partners, anal intercourse), presence of vulvar intraepithelial neoplasia (VIN) or cervical intraepithelial neoplasia (CIN) were abstracted from chart review. Descriptive statistics and univariate analyses were used to compare women with and without HGAIN (chi-square test for categorical variables, 2-sample t test for continuous variables).

Results

There were 55 women identified during the study period, of which 22 were found to have HGAIN. Women with HGAIN had a significantly greater smoking history (mean pack-years: 23.8 vs 7.6, $p=0.003$) and were more likely to have VIN 2-3 ($p=0.032$). History of immunosuppressive state was marginally non significant ($p=0.051$). None of the other variables including age at coitarche, number of sexual partners and history of anal intercourse were significantly different between groups.

Conclusion

We found that heavy smoking and high grade VIN were associated with presence of HGAIN. Further study in a larger cohort to confirm our findings is needed to identify high risk women who should be screened for HGAIN using HRA.

SS 13-04

Human papilloma virus 35 is an aggressive subtype in long term follow up of equivocal (ASCUS) and low grade (LSIL) HPV positive cervical smears in Western Norway.

O.K. Vintermyr¹, T. Bjørge², R. Skar³, O.E. Iversen⁴, H.K. Haugland⁵

¹Department of Pathology, Haukeland University Hospital, Bergen. The Gade Laboratory for Pathology, Department of Clinical Medicine, University of Bergen, Bergen (Norway), ²Department of Global Public Health and Primary Care, University of Bergen, Bergen. (Norway), ³Department of Global Public Health and Primary Care, University of Bergen, Bergen. Cancer Registry of Norway, Oslo. (Norway), ⁴Department of Clinical Medicine, University of Bergen, Bergen. Women's Clinic, Haukeland University Hospital, Bergen. (Norway), ⁵Department of Pathology, Haukeland University Hospital, Bergen. (Norway)

Background / Objectives

A retrospective, population based study from Western Norway among women with equivocal (ASCUS) or low grade (LSIL) cell changes in the period 2006-2009 and with a positive HPV test in their first follow up (control) test. Women with prior (last two years) abnormal smear(s) and/or positive HPV tests, or lack of follow-up information were excluded.

All women were followed for 5-9 years with respect to progression (CIN2+/CIN3+) or remission.

Methods

The high risk (hr)HPVsubtyping was done on stored Hybrid Capture (HC2) samples. For hrHPV subtyping a commercial multiplex PCR (f-HPV, Genomed LTD, UK) test was used that identifies essentially the same hrHPV subtypes as in HC2. A few samples with no definite HPV subtype in the f-HPV test were re- tested using standard Gp5+/GP6+ HPV PCR primers followed by DNA (Sanger) sequencing for HPV type identification.

A relative hrHPV subtype estimate for risk of progression to CIN2+/CIN3+ was defined as the fraction of women with a specific hrHPV subtype that progress to CIN3+ versus the fraction that regress x 100 during the period of observation. The relative hrHPV subtype risk was compared with hrHPV negative and hrHPV negative/cytology negative (normal) women in a similar screening population cohort (1).

Results

A total of 804 women were HPV tested (16 samples were missing) and in 753 of these (93.7%) a specific hrHPV subtype (single or multiple) could be determined. Among these, only 61 women (7.4%) did not meet pre-set criteria for remission or progression by the end of 2014. The prevalence of HPV subtypes, in decreasing order, among women undergoing progression (CIN2+) and regression were: HPV16 (49%), 33 (18%), 52 (15%), 51 (9%), 35 (8%), 58 (8%), 39 (7%), 18 (6%), 31 (5%), 59 (5%), 45 (5%), 68 (3%), 56 (2%), 66 (1%) and HPV 16 (27%), 33 (12%), 52 (11%), 39 (11%), 58 (10%), 51 (9%), 18 (7%), 59 (6%), 56 (6%), 68 (5%), 45 (5%), 35 (5%), 31 (4%), 66 (3%), respectively. Relative hrHPV subtype progression risk to CIN 2+/CIN3+ was determined with respect to hrHPV type, dominant hrHPV type, single hrHPV types, and multiple hrHPV infections. HPV 16, 31, 33 and 35 were the most potent hrHPV types. For single hrHPV infections HPV 35 was as potent as HPV 16 for progression into CIN2+/CIN3+.

Conclusion

hrHPV 16 was the dominant HPV type in women undergoing progression and regression in delayed triage of HPV positive ASCUS/LSIL. HPV 35 was found in 5% of the lesions undergoing remission and in 8% of lesions progressing to CIN2+. For single hrHPV infections HPV 35 was as potent as HPV 16 to progress into high risk lesions.

References

Budal EB, et al. HPV DNA testing improves CIN2+ risk stratification and detection of CIN2+ in delayed triage of ASCUS and LSIL. A population-based follow-up study from Western Norway. *Cancer Med.*, 2014, 3:182-9.

SS 13-05

Are patients with a first potentially-human papillomavirus-related cancer at greater risk of second primary cancer? a French population-based study

F. Neumann, A.S. Woronoff

Registre des tumeurs du Doubs et du Territoire de Belfort (France)

Background / Objectives

Human papillomaviruses (HPV) are involved in the development of anogenital and head and neck cancers. The purpose of this study was to assess the risk of developing a second primary cancer (SPC) after a first potentially-HPV-related cancer.

Methods

All patients with a first cancer diagnosed between 1989 and 2004, as recorded by 10 French cancer registries, were followed up until December 31, 2007. Only invasive potentially-HPV-related cancers (namely, cervical, vagina, vulva, anal canal, penile, oropharynx, tongue and tonsil) were included. Standardized Incidence Ratios (SIRs) were calculated to assess the risk of SPC in these patients compared with the general population. A multivariate Poisson regression model was used to model SIRs separately by gender, adjusted for the characteristics of the first cancer.

Results

10,127 patients presented a first potentially-HPV-related cancer. The overall SIR was 2.48 (95% CI, 2.34-2.63). The SIR was 3.59 (95%CI, 3.33-3.86) and 1.61 (95%CI, 1.46-1.78) in men and women respectively. The relative risk of potentially-HPV-related SPC was high among these patients (SIR=13.74; 95%CI, 8.80-20.45 and 6.78; 95%CI, 4.61-9.63 for men and women, respectively). Women diagnosed in the most recent period (2000-2004) showed a 40% increase of their relative risk of SPC as compared with women diagnosed between 1989 and 1994 (ratio of SIRs=1.40; 95% CI, 1.06-1.85).

Conclusion

HPV cancer survivors face an increased risk of SPC, especially concerning second cancer sites potentially related to HPV. Clinicians may consider this increased risk of developing HPV-related SPC during follow-up to improve subsequent cancer prevention in these patients.

SS 13-06

DOES A HISTORY OF CHILDHOOD UNWANTED SEXUAL EXPERIENCES INFORM SEXUAL ORIENTATION AND RELATIONSHIPS WITH SAME-SEX PARTNERS?

A. Subasinghe¹, Y. Jayasinghe², J. Wark³, A. Gorelik⁴, S. Garland⁵

¹Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Murdoch Childrens Research Institute (Australia), ²Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Department of Obstetrics and Gynaecology, University of Melbourne (Australia), ³Department of Medicine, University of Melbourne, Royal Melbourne Hospital (Australia), ⁴Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Melbourne EpiCentre, Royal Melbourne Hospital, Melbourne (Australia), ⁵Department of Microbiology and Infectious Diseases, Royal Women's Hospital, ³Department of Obstetrics and Gynaecology, University of Melbourne (Australia)

Background / Objectives

The relationship between childhood unwanted sexual experiences (USEs) and sexual orientation has yet to be investigated in Australian females aged below 18 years.

Methods

Women aged 16-25 years living in Victoria were randomly recruited via targeted advertising on Facebook from May 2010. A web-based questionnaire was used to collect information on participant demographics, USEs and indices of sexual orientation. Chi2 tests and multivariable logistic regression were used to determine associations between a history of childhood and adulthood USE and indices of sexual orientation.

Results

Data were collected from 639 females (mean \pm SD age 22 \pm 3). Approximately 14% had experienced childhood USE and 15% reported adult USE. Females who reported to have a same-sex sexual orientation (N=23, 4%) were more likely to have experienced childhood USE than those who reported to be heterosexual (OR 4.6, 95% CI 1.7–12.1, $p < 0.001$). Childhood USE was associated with a greater likelihood of having at least one female partner compared to those who had not reported childhood USE (OR 2.5, 95% CI 1.3–4.6, $p = 0.004$). A similar association was detected between those who reported adulthood USE and having a female partner.

Conclusion

Using a novel web-based validated questionnaire, we found that the prevalence of childhood and adulthood USE is extremely high. A positive association between childhood USE and same-sex sexual orientation was found, but we were unable to determine whether the USE preceded sexual orientation. Longitudinal studies should be conducted in Australian females to investigate whether these associations are bi-directional.

SS 13-07

CHARACTERIZATION OF SEXUAL HEALTH BEHAVIOURS AMONG YOUNG WOMEN LIVING IN VICTORIA, AUSTRALIA

N. Nguyen¹, J. Wark², A. Subasinghe³, S. Garland⁴

¹Department of Medicine, University of Melbourne (Australia), ²Department of Medicine, University of Melbourne, Royal Melbourne Hospital, (Australia), ³Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Murdoch Childrens Research Institute, (Australia), ⁴Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne (Australia)

Background / Objectives

Young females aged 16–25 years are at high risk of sexually transmissible infections (STIs) predominantly contracted through unsafe sexual practices. There is also evidence from previous studies to show an association between psychological distress and risky sexual behaviours. We aimed to investigate the association between psychological distress and risky sexual behaviors in young females aged 16-25 living in Victoria, Australia.

Methods

Data were extracted from a cross sectional study called the Young Female Health Initiative (YFHI). Participants were recruited via advertisements on Facebook from 2012. Risky sexual behaviors (outcomes) were measured through five binary variables: sex while drunk or high, sex with a non-current partner, having more than one male partner in a lifetime, paid sex, and non-current use of condom. Psychological distress was measured using the Kessler Psychological Distress Scale (K10). Logistic regression models were used to estimate unadjusted odd ratios (ORs) and adjusted ORs.

Results

We detected a significant association between Kessler score and sex while drunk or high (OR 1.7, 95% confidence interval (CI) 1.2, 2.7, $p=0.006$). After adjusting for covariates, the estimated relative change in the odds of having sex while drunk or high increased minimally to 1.8 (95% CI 1.1, 3.0, $p=0.02$) for a change in Kessler score of 7 units.

Conclusion

Our findings suggest that young females engaging in risky sexual behaviours should be screened for psychological distress. Further studies should be conducted to assess the temporal association between psychological distress and risky sexual behaviours.

SS 14-02

HPV negative cervical cancer at the ICO survey: Interpretation and impact

S. De Sanjose

Catalan Institute of Oncology (Spain)

Background / Objectives

The current view on HPV contribution on cervical cancer is that 100% of the cases are attributable to HPV persistent infection. However, this absolute presence is rarely seen in the field studies or in the clinical settings. Concern of potential false negatives cases has become more worrisome due to the upcoming screening algorithms using HPV tests as primary selection process in cervical cancer screening.

Methods

Histological confirmed ICC cases from 38 countries were assembled. HPV detection was done by polymerase chain reaction using SPF-10 broad-spectrum primers followed by deoxyribonucleic acid enzyme immunoassay and genotyping by reverse hybridization line probe assay (LiPA25) (version 1). Several steps were taken to evaluate the potential reasons for a false negative result: HPV DNA negative samples were evaluated for DNA quality for PCR analysis by the application of different primers for β -globin and β -Actin PCR amplification generating different amplicon lengths. Besides broad spectrum PCR we also tested with 14 different hr HPV Type Specific PCRs. Adenocarcinomas were reviewed by a panel of expert pathologists

Results

Of 10,575 ICC cases, 8,977 were HPV-DNA positive (84.9%). The diagnosis with the lowest HPV positivity was ADC (61.8%), followed by the "other" category (72.5%) and ADSCC (81.2%). Among HPV DNA negative samples 60% were also β -globin and β -Actin PCR negative. In the remaining 40% of the HPV negative samples showed no signs of PCR inhibition after spiking these samples with HPV DNA and performing HPV type specific tests. Additional evaluation of these HPV negative specimens both in the quality of the specimen and the pathology processing as well as in the HPV testing methodology indicated that low viral load, tissue degradation could account for less than 10% of negative ADC. Misdiagnosis of ADC was not identified as playing a major role. Preservation of the tissue may be related to unmeasured factors that could explain results. Further analysis will be presented to explore factors linked to a negative result.

Conclusion

Several issues can be involved in a false negative HPV result when using repository samples. The impact of them is unlikely to affect the genotype distribution of the positive samples. False negative data can be minimized when using recent and well preserved samples.

SS 14-05

Sensitivity of HPV testing vs HPV & cytology co-testing in primary screening using cervical cancer as the outcome: a meta-analysis

M. Arbyn

Belgian Cancer Centre / Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels (Belgium)

Background / Objectives

Recent reports from large cytopathology laboratories in USA and China, linking prior testing of cervical cell specimen with the subsequent occurrence of invasive cervical cancer revealed significantly higher false-negativity rates for HPV testing than for the combination of HPV testing and cytology. The authors concluded that co-testing should be the preferred screening policy.

Methods

Prior meta-analyses on the accuracy of HPV and cervical cytology to detect underlying CIN2+ or CIN3+ were updated and extended for the outcome of invasive cervical cancer. The current report assesses the sensitivity of high-risk HPV testing and compares it with that of co-testing. Standard random effects models for pooling of binomial test accuracy data were used.

Results

The sensitivity pooled from 16 studies was 85% (95% CI: 80-90%, I²=26%, p for heterogeneity<0.001) and 95% (95% CI: 91-99%, I²=84%, p<0.001) for hrHPV testing and co-testing, respectively. Co-testing was 11% more sensitive than hrHPV (relative sensitivity=1.11 [95% CI: 1.08-1.15]). The absolute sensitivity varied by test and interval between test and cancer diagnosis (p<0.001), however relative sensitivity did not show significant heterogeneity (p=0.26).

Conclusion

Although the meta-analysis confirms the aforementioned reports, flaws on the recent linkage studies should be noted on design and conclusions. Linkage studies should only include screening histories and exclude recent tests immediately preceding a diagnosis of cancer. Moreover, sensitivity estimates for cancer are not the most relevant criterion for decision making. Using the KPNC

screening, we estimated a NPV for cervical cancer of 99.995% vs 99.998% of hrHPV vs cotesting, respectively. This would correspond with ~three additional cases of cancer detected but also ~4,500 more false-positive cases per 100,000 screened with co-testing compared to a general hrHPV test only.

In a HPV-based cervical cancer screening, an extensive virological audit of archived specimen from each HPV-negative case of cancer case should be set up.

SS 15-01

Introduction to HPV Frame

K. Canfell

Cancer Council New South Wales, The University of Sydney (Australia)

Background / Objectives

HPV-FRAME is an initiative to develop a consensus statement and quality framework for modelled evaluations of HPV prevention. This talk will describe the history and rationale of the initiative and will discuss then progress to date and next steps.

Methods

Quality assessment of HPV models will be structured according several which reflect the policy questions of interest in HPV prevention. For each of these domains, a draft framework will be presented by members of the HPV-FRAME coordinating group at EUROGIN 2014. An opportunity for public comment will follow and the framework will eventually be published and disseminated.

Conclusion

The initiative will allow for the development of models in accordance with an explicit reporting and quality framework. This will allow the end-user, often a policy-maker, to appreciate how accurately the model reflects outcomes prior to change, the areas of simplification, whether a model construction and parameterization was appropriate to the decision question and the degree of uncertainty in a decision process.

SS 15-02

HPV-FRAME and general guidelines for good modelling practice: How do the two relate?

M. Brisson

2. Département de médecine sociale et préventive, Université Laval, Québec, Canada (Canada)

Background / Objectives

HPV-FRAME will be a quality framework which will enable a set of standards that ensure models of HPV prevention contribute to an optimal decision-making process.

Methods

As background to the specific discussion of HPV models to occur in this session, this talk will 'set the scene' via discussion of the general principles of good modelling practice as articulated in the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines for good modelling practice, which include general principles for model calibration, validation and uncertainty and sensitivity analysis.

Conclusion

HPV-FRAME will supplement general principles of good modelling practice as articulated by ISPOR, via the provision of specific guidance on issues of relevance in HPV modelling.

SS 15-03

Presentation of draft framework: General principles for models of universal HPV vaccination in females and/or males

M. Jit

London School of Hygiene & Tropical Medicine (United kingdom)

Background / Objectives

Thirteen years have passed since the publication of the first models of HPV vaccination. Since then around a hundred economic models of HPV vaccination have been published, along with many models presenting epidemiological but not economic outcomes. An array of methodological options are available to model HPV vaccination and several best practice guidelines have been published, but are still not well known or adhered to.

Methods

Previous frameworks and guidelines for modelling of HPV vaccination were reviewed. A comprehensive set of reporting requirements were drafted with input from other members of the HPV-FRAME consortium

Conclusion

The draft HPV-FRAME reporting requirements for parameters, calibration targets and validation outputs for both female-only and gender-neutral models of universal HPV vaccination will be presented. There will be the opportunity to give feedback both during the session and afterwards, to be taken into account in future iterations of the draft framework.

SS 15-04

Presentation of draft framework: Additional issues for models of targeted HPV vaccination in MSM

M. Jit

London School of Hygiene & Tropical Medicine (United kingdom)

Background / Objectives

Men who have sex with men (MSM) have a disproportionately high burden of HPV-related diseases but obtain little indirect benefit from female-only vaccination. Older MSM also do not receive much benefit from male vaccination programmes targeting younger adolescents. Hence targeted HPV vaccination for men who have sex with men is an option that has been implemented in several countries alongside either female-only or gender-neutral programmes. However, the feasibility and cost-effectiveness of such a programme is a key consideration for countries.

Methods

The draft reporting requirements for models of HPV vaccination were extended to targeted MSM programmes with input from other members of the HPV-FRAME consortium.

Conclusion

The additional draft HPV-FRAME reporting requirements for parameters, calibration targets and validation outputs for models of targeted vaccination in MSM will be presented. There will be the opportunity to give feedback both during the session and afterwards, to be taken into account in future iterations of the draft framework.

SS 15-05

Presentation of draft framework: Models of alternative vaccine types and reduced-dose schedules

J. Kim

Harvard TH Chan School of Public Health, Boston, MA (United States of America)

Background / Objectives

As part of the Framework for Modeling HPV Prevention (HPV-FRAME) consortium, general principles for models of universal HPV vaccination in females and/or males will be presented. This talk will present the additional draft HPV-FRAME reporting requirements for parameters for comparative evaluations of different vaccine types and/or reduced dose schedules.

Methods

We identified distinct parameters that are critical to specify in comparative model-based analyses of alternative vaccines including the differential levels of vaccine cross-protection (i.e., level and duration of protection against specific types), cost (per dose) and total cost per vaccinated girl for each vaccine, assumptions regarding type replacement in the model, and any assumptions regarding differential uptake and/or availability of the vaccines. For alternative dosing schedules, important specifications include efficacy and duration of protection against specific types, assumed schedule between more than 1 dose, target age groups for reduced dose schedule, cost (per dose) and total cost per vaccinated girl for 1-dose or 2-dose schedules, assumptions regarding cross-protection for less than three doses, and assumptions regarding differential uptake and scale-up for policies involving different dosage schedules.

Conclusion

Clear and consistent reporting of inputs and assumptions in evaluations comparing different HPV vaccines and alternate vaccine dosing schedules are critical for interpreting results, assessing quality of analyses, and facilitating comparisons across studies.

SS 15-07

HPV Frame presentation of draft framework: Models of integrated cervical screening and vaccination approaches

J. Berkhof

VU University Medical Center Amsterdam (Netherlands)

Background / Objectives

Cost-effectiveness modelling is essential to support decision making relating to screening in vaccinated populations. HPV-FRAME will provide reporting requirements for HPV modelling studies.

Methods

Additions to the HPV FRAME reporting requirements for screening in vaccinated populations will be presented. Key issues are data sources presently used for integrated vaccination-screening strategies (randomized screening trials in vaccinated women, screening trials and cohort studies with HPV genotyping, and screening and vaccination registries), calibration targets, screening of cohorts offered first or second generation vaccines, vaccination and screening uptake, and risk based screening.

Conclusion

In the coming years, vaccinated women will become screen-eligible and a timely cost effectiveness assessment is important when preparing an integrated vaccination-and-screening programme. An itemized reporting checklist for modelling studies will provide guidance and support good modelling practice.

SS 15-08

Presentation of draft framework: HPV-FASTER evaluations

K. Canfell

Cancer Council New South Wales, The University of Sydney (Australia)

Background / Objectives

As part of the Framework for Modeling HPV Prevention (HPV-FRAME) consortium, general principles for models of HPV vaccination and for integrated models of vaccination and cervical screening will be presented. This talk will present the additional draft HPV-FRAME reporting requirements for parameters for evaluations of 'HPV-FASTER' strategies which are broadly defined as including HPV vaccination in older women and/or men (>20-26 years) and/or combined vaccination/screening strategies where vaccination is performed in older women.

Methods

We identified distinct parameters and assumptions that are critical to specify in model-based analyses of vaccination with or without screening in women and men aged over 20-26 years. In addition to the general requirements for reporting on models of screening or integrated screening and vaccination strategies, modelling of 'HPV-FASTER' options should report assumptions on HPV exposure at older ages as well as the age-and type- specific assumptions about the probability of disease progression in individuals infected with HPV at older ages. The calibration and reporting on outputs for natural history models of disease at these relatively older ages will be a key focus. The role of threshold analysis on vaccine price will be discussed.

Conclusion

There is emerging interest in delivering HPV vaccination to older individuals, but this poses challenges for models since the population-level effectiveness and cost-effectiveness of such strategies depends not only on vaccine delivery methods and costs but also, critically on the modelling of HPV exposure (incidence) and persistence in older people. Given the probability of HPV exposure and/or progression is lower in older people than in unvaccinated adolescents, vaccination at older ages (at a given vaccine price) is less likely to be cost-effective than it is for pre-adolescents. Adequate reporting on modelling of the natural history of HPV infection in older individuals will enable assessment of quality of the analysis, and to facilitate comparison across studies.

SS 15-09

Presentation of draft framework: Additional issues for models of HPV prevention in low and middle income countries

J. Kim

Harvard TH Chan School of Public Health, Boston, MA (United States of America)

Background / Objectives

As part of the Framework for Modeling HPV Prevention (HPV-FRAME) consortium, general principles for models of HPV vaccination and for models of cervical screening will be presented. This talk will present the additional draft HPV-FRAME reporting requirements for parameters for evaluations of HPV prevention in low- and middle-income countries (LMIC).

Methods

We identified distinct parameters and assumptions that are critical to specify in model-based analyses of vaccination and/or screening in LMIC. In addition to the general requirements for reporting on country- or setting-specific information on burden of infection and disease, analyses in LMIC should report assumptions of the feasibility of implementation and sustainability of screening or vaccination programs, as well as the data that inform these assumptions. This includes scenarios of current "status quo" practice (e.g., existing immunization programs and existing screening programs), as well as specification of additional constraints that may inhibit implementation and scale-up of intervention(s). Analyses of vaccination should report on the assumed vaccine target population(s) and corresponding delivery mechanism. Budget or cost effectiveness evaluations should specify currency (and year) for costs, as well as the method of cost inflation and/or conversion, for example, using purchasing power parity index for comparisons across settings and assumptions of tradeable versus non-tradeable goods. The willingness to pay threshold for a particular country should be clearly specified and justified.

Conclusion

Analyses in the context of LMIC should be held to the same reporting standard as those for high income countries (specified in the general principles for models of HPV vaccination and cervical screening). In addition, there are inputs that are unique to analyses in LMIC that are also important

to disclose to ensure proper interpretation of results, to enable assessment of quality of the analysis, and to facilitate comparison across other studies.

GS 01-04

Krebsentstehung durch HPV

M. Von Knebel Doeberitz

Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, (Germany)

Background / Objectives

Humane Papillomaviren können verschiedenste Läsionen des Plattenepithels hervorrufen. Mittlerweile kennt man fast 200 verschiedene Papillomvirustypen, die entweder das verordnende Plattenepithel der Haut oder das nicht-verhornende Plattenepithel der Schleimhäute infizieren können. Die überwiegende Mehrheit der HPVs verursacht bei gesunden Personen keine Läsionen von klinischer Bedeutung. Vor allem die Typen 6 und 11 können jedoch zu z.T. grosse Läsionen auf den Schleimhäuten (condylomata accuminata) hervorrufen, die jedoch in aller Regel benigne bleiben. Eine Gruppe von weiteren etwa 14 HPVs werden als potentiell onkogen eingestuft, da sie häufig in HPV-assoziierten prämaligen und malignen Läsionen gefunden werden. Hierbei sind HPV 16 und 18 die absolut prädominanten Typen, da sie alleine in etwa 70 bis 80 % aller HPV-assoziierten Malignome gefunden werden. HPV-assoziierte Tumoren weisen eine charakteristische Abhängigkeit von 2 viralen Onkogenen, die als E6 und E7 bezeichnet werden auf (oncogene addiction). Die Expression dieser beiden Gene in HPV-infizierten Zellen führen zu einer chromosomalen Destabilisierung und in der Folge zur malignen Transformation und Progression. Interessanterweise tritt die Aktivierung dieser beiden Onkogene vorzugsweise in hochspezialisierten Epithelzellen, den sog. squamous columnar junction cells an der Transformationszone der Cervix uteri sowie im Bereich der Linea dentata im Analkanal oder aber auch im Bereich des Waldeyer-Epithels des Oropharynx auf. Der Lebenszyklus der Papillomaviren wird offenkundig durch das eigenetische Milieu der Wirtszellen entscheidend mit bestimmt. So scheint auch die Expression der viralen Onkogene durch eigenetische Mechanismen mit reguliert zu sein. Ein Behandlungsversuch von zahlreichen HPV-transformierten Zelllinien mit demethylierenden Substanzen zeigte, dass in allen bisher untersuchten Zelllinien die Expression und Funktion der viralen Onkogene gehemmt wird. Dies führt zum Wachstumsarrest und Absterben der Zellen in vitro. Basierend auf diesen experimentellen Beobachtungen testen wir derzeit in klinischen Studien, ob durch demethylierende Substanzen möglicherweise auch eine zielgerichtete Therapie HPV-assoziiierter Neoplasien bei Patienten realisierbar ist.

Methods

na

GS 01-05

EPIDEMIOLOGIE VON ANOGENITAL-TUMOREN

N. Buttman-Schweiger, K. Kraywinkel

**Robert Koch-Institut, Abteilung Epidemiologie und Gesundheitsmonitoring,
Zentrum für Krebsregisterdaten (ZfKD), Berlin (Germany)**

Background / Objectives

Als Beitrag zu einer umfassenden Begleitforschung zur Wirksamkeit der Primär- und Sekundärprävention HPV-attributabler Krebserkrankungen stellen wir bundesweite aktuelle Ergebnisse und zeitliche Trends aus den Daten der etablierten bevölkerungsbasierten Krebsregister in Deutschland vor.

Methods

Identifikation von Karzinomen des Anus, der Zervix und des äußeren Geschlechts (AGT) von Männern und Frauen mit Diagnosezeitpunkt 1999-2012 aus 10 der bevölkerungsbezogenen Krebsregister Deutschlands. Inzidenzraten wurden altersstandardisiert nach altem Europastandard; das relative 5-Jahres-Überleben basiert auf Grundlage der bundesdeutschen Periodensterbetafeln und ist nach der Ederer-II-Methode berechnet [1]. Die durchschnittliche jährliche Veränderung der altersstandardisierten Inzidenzraten über den 13-Jahres Zeitraum wurde mit Regressions-Modellen in Joinpoint berechnet.

Results

Im Jahr 2012 erkrankten 10.122 Frauen und 2.614 Männer an AGT. Das Alter bei Diagnose hat entscheidenden Einfluss auf die weitere Prognose der Erkrankung. Ein Viertel aller betroffenen Frauen und 12.1% der Männer war bei Diagnose unter 50 Jahre. An Gebärmutterhalskrebs erkrankten Frauen im Mittel bereits mit 53 Jahren. Etwa ein Drittel der Frauen verstarb in den ersten 5 Jahren nach Diagnose an Ihrem Krebsleiden (relatives 5JÜ: 67,5%). In Deutschland leben aktuell 32.534 Frauen, die innerhalb der letzten 10 Jahre mit Gebärmutterhalskrebs diagnostiziert wurden. Frauen mit AGT (ohne Zervix) und Männer mit AGT erkrankten im Mittel mit 71 bzw. 69 Jahren; ihr relatives 5JÜ lag bei 66,3% bzw. 65,3%. Die Inzidenz- und Mortalitätsraten des Zervixkarzinoms bleiben seit 2004/2005 konstant. Hingegen steigt, ähnlich der Neuerkrankungsraten der Vulva (+6,7%; 95%CI 5,6-7,9) und Analkarzinome der Frau (+3,4%; 95%CI 2,3-4,5), auch die Inzidenzrate der AGT bei Männern seit 1999 stetig um 2,1% (95%CI 1,6-2,7) pro Jahr. Auf Grundlage attributabler Anteile schätzen wir, dass jährlich etwa 6.000 der weiblichen Fälle und 760 der männlichen AGT-Fälle einer Infektion mit HPV zuzuschreiben sind.

Conclusion

Die Neuerkrankungs- und Sterberaten durch Gebärmutterhalskrebs sind seit Mitte der 2000er weitgehend stabil. Im Gegensatz hierzu haben die Karzinome der äußeren Genitale, die Scheiden- und Analkarzinome, im letzten Jahrzehnt jährlich zugenommen. Das Monitoring auf Grundlage von Krebsregisterdaten kann nur ein Baustein einer weiteren umfassenden Begleitforschung sein. Entsprechende Datenflusskonzepte zwischen den Krebsregistern und dem Screeningprogramm können zukünftig dazu beitragen, Ergebnisse von konsentierten Surrogatparametern und Endpunkten in Auswertungen zusammenzuführen und mögliche Effekte abzuschätzen.

References

1. Krebs in Deutschland 2011/2012. 10. Ausgabe. Robert Koch-Institut (Hrsg) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg). Berlin, 2015

GS 01-07

NATÜRLICHER VERLAUF DER HPV INFEKTION

F. Stubenrauch

Universitätsklinikum Tübingen (Germany)

Persistierende Infektionen mit den high-risk HPV (HR-HPV) Typen 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 und 59 sind für die Entstehung von Gebärmutterhalskarzinomen und anderen ano-genitalen Karzinomen verantwortlich. HPV besitzen ein kleines, doppelsträngiges DNA-Genom, das nur ca. zehn virale Proteine kodiert, und sind daher auf zelluläre Replikationsfaktoren für die Virusvermehrung angewiesen. HPV infizieren nach Mikrowunden oder Traumen Keratinozyten in der Basalzellschicht der verhornenden Haut oder Schleimhaut. Der virale Replikationszyklus hängt vom Differenzierungszustand der infizierten Keratinozyten ab und kann in organähnlichen Zellkulturmodellen analysiert werden. HPV werden nach Bindung an Rezeptoren auf der Wirtszelle internalisiert und das Genom wird in den Zellkern transportiert. Die Expression von viralen Regulatorproteinen führt zu einer begrenzten Vermehrung der Virusgenome in basalen und suprabasalen Keratinozyten. Die viralen Onkoproteine E6 und E7 induzieren in suprabasalen Zellen den Wiedereintritt in den Zellzyklus und aktivieren so die zelluläre und virale DNA Replikation. Gleichzeitig mit der Amplifikation der viralen Genome in suprabasalen Zellen kommt es zur Aktivierung des späten viralen Promoters, was in terminal differenzierten Keratinozyten zur Expression der viralen L1 und L2 Kapsidproteine und somit zu infektiösen Viruspartikeln führt. Neuere Daten weisen überraschenderweise daraufhin, dass die Replikation von HR-HPV Genomen in suprabasalen Zellen eine Aktivierung von DNA-Schädigungs-Signaltransduktionswegen („DNA Damage Response“) und zelluläre DNA-Rekombinationsproteine benötigt. Auf der Basis dieser Befunde werden Modelle zur Progression von produktiven HR-HPV Läsionen zu Zervixkarzinomen diskutiert.

GS 02-01

Grundlagen der prophylaktischen HPV Vakzine

C. Schellenbacher

Abt. f. Immundermatologie und Infektiöse Hautkrankheiten, Univ. Klinik f. Dermatologie, Medizinische Universität Wien (Austria)

Background / Objectives

Die zugelassenen Vakzinen gegen Humane Papillomviren (HPV) sind prophylaktisch wirksame Spaltimpfstoffe aus dem Hauptvirkuskapsidprotein L1. Dieses bildet spontan Virus-ähnliche Partikel (L1-VLP), die morphologisch und immunologisch dem nativen infektiösen Virus ähneln, jedoch keine virale DNA enthalten und nicht replizieren können. Immunisierung mit L1-VLP induziert hoch-titrige und lang persistierende neutralisierende Antikörper gegen konformationelle und Typen-spezifische Epitope von L1.

Methods

Die beiden Impfstoffe der ersten Generation, Cervarix™ und Gardasil™, enthalten neben Adjuvantien L1-VLP der häufigsten genitalen hoch-Risiko HPV 16 und HPV 18, die 70% aller Zervixkarzinome, 90% aller Analkarzinome und einen geringeren Prozentsatz anderer anogenitaler und oropharyngealer Karzinome verursachen. Gardasil enthält zudem L1-VLP der niedrig-Risiko HPV 6 und HPV 11, die 90% aller Genitalwarzen verantworten. Die Effizienz der Vakzinen liegt bei fast 100% gegen persistierende Infektionen und Erkrankungen, hervorgerufen durch die von den Impfstoffen abgedeckten HPV Typen, sofern sie prophylaktisch verabreicht werden. Klinische Studien zeigten die Wirksamkeit der L1-VLP Impfstoffe gegen zervikale, vulväre, vaginale und anale intraepitheliale Neoplasien und gegen Genitalwarzen. Klinische Daten, die nach Impfstoffzulassung in einer Gesamtpopulation (Australien) mit hoher Impftrate in jungen Frauen erhoben wurden, belegen die hohe Wirksamkeit der L1-VLP Impfstoffe anhand der dramatischen Reduktion von Genitalwarzen innerhalb weniger Jahre. Daten aus klinischen Studien und Impfregistern sprechen für eine hohe Sicherheit der zugelassenen L1-VLP Impfstoffe.

Conclusion

Limitierungen der derzeit erhältlichen Vakzinen sind die vergleichsweise hohen Kosten und die Beschränkung des Impfschutzes auf die hoch-Risiko HPV16 und 18, die 70% der Zervixkarzinome verursachen, während 30% der Fälle durch >15 weniger häufige hoch-Risiko HPV (z.B. HPV 31, 33, 45, 52, 58,...) verursacht werden. Erstere können durch neue Impfschemen mit weniger als 3 Impfdosen reduziert werden. Zur Erweiterung des Impfspektrums wurde 2015 ein nonavalenter Impfstoff (Gardasil-9) zugelassen, der durch Inklusion von L1-VLP weiterer 5 hoch-Risiko HPV einen Impfschutz gegen 90% aller Zervixkarzinome verspricht. Alternative experimentelle Ansätze einer Breitspektrum HPV Vakzine auf Basis von hoch-konservierten Epitopen des Nebenkapsidproteins L2 sind in klinischer Entwicklung.

GS 02-02

Update zur Impfung gegen HPV - nonavalenter Impfstoff

S. Pils, E. Joura

Medical University Vienna (Austria)

Background / Objectives

Seit Dezember 2014 ist in den USA der nonavalente HPV-Impfstoff (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58) zugelassen. Die europäische Zulassung für Frauen und Männer ab 9 Jahren erfolgte im Juni 2015. Ab Mitte 2016 ist dieser Impfstoff (Gardasil9[®], Sanofi Pasteur MSD, Lyon Frankreich) in Europa erhältlich.

Methods

Es wird ein Überblick über die Zulassungsstudien des nonavalenten HPV-Impfstoffes und deren Schlüsselresultaten gegeben.

Results

Im Vergleich zum quadrivalenten HPV-Impfstoff (HPV 6, 11, 16, 18, Gardasil[®], Sanofi Pasteur MSD, Lyon Frankreich) bietet der nonavalente Impfstoff eine ca. 97% Reduktion von Infektionen und Erkrankungen durch die HPV Stämme 31, 33, 45, 52 und 58. Weiters ist die Immunogenität und die klinische Schutzwirkung gegenüber Infektionen und Erkrankungen mit den HPV Stämmen 6, 11, 16 und 18 gleich wie bei dem quadrivalenten Impfstoff. Die Immunogenität bei Mädchen und Buben von 9 bis 15 Jahren ist stärker als bei Frauen im Alter von 16 bis 26 Jahren, die in der klinischen Zulassungsstudie untersucht wurden. Außerdem wurde eine gute Immunogenität bei jungen Männern bis 26 Jahren nachgewiesen, ebenso ist eine Wirksamkeit bei einem 2 Dosis Schema zu erwarten. Das Sicherheitsprofil der Impfung ist als günstig zu bewerten.

Conclusion

Der nonavalente HPV Impfstoff erzeugt einen typenspezifischen und daher sicheren Schutz gegenüber den 7 wichtigsten onkogenen HPV Stämmen die ca. 90% aller HPV assoziierten Karzinome verursachen. Durch eine frühe, geschlechtsneutrale Impfung und hoher Durchimpfungsrate wird eine

dramatische Reduktion von Karzinomen und Krebsvorstufen mittel bis langfristig zu erwarten sein. Die Immunogenität des Impfstoffes bei Frauen von 27 bis 45 Jahren ist noch zu untersuchen.

References

Joura EA, Giuliano AR, Iversen OE, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N Engl J Med* 2015;372:711-23.

Pils S, Joura EA. From the monovalent to the nine-valent HPV vaccine. *Clin Microbiol Infect.* 2015;21:827-33.

GS 03-01

Updated Munich Nomenclature III for Cervical Cytology

H. Griesser

Laboratory Dr. Wisplinghoff, Dept. Pathology and Cytodiagnostics, Cologne (Germany)

Background / Objectives

The Munich Nomenclature II, since 1990 officially in use for the German national cancer screening program, needed an update to account for the accumulated knowledge about the biology and epithelial cell transformation potential of HPV infection, to better represent glandular lesions in the cytological grading system, and to be translatable into the Bethesda System (TBS). Especially, ASC-US cytology had not been accounted for in the old grading system, thus reducing sensitivity of the cytological approach to detect potentially precancerous conditions of the cervix.

Methods

Members of the Coordination Conference for Cytology (delegates of the German professional and scientific societies for cytology, pathology and gynecology) drafted the updated Munich Nomenclature III (MN III), taking into consideration the continuity of the previous German grouping system and the traditional separation of mild (group IIID1), moderate (group IIID2) from severe dysplasia (IVa-p). In order to estimate the distribution of different diagnostic categories within MN III, statistical evaluation was performed for the results of five cytology labs across Germany based on 287,346 women over a six months period.

Results

The referral labs did not encounter difficulties switching from MN II to MN III and altogether had a high NILM rate (Group I and II-a: 97.6 %), a low rate of ASC-US or ASC-H (II-p, 0.6% or III-p, 0.2%). LSIL (IIID1) rate was 0.7%, HSIL 0.6% (IIID2 and IVa-p/IVb-p: 0.4 and 0.2%). The rate of possible or precancerous glandular lesions (AGC (II-g or III-g) / AIS (IVa-g/IVb-g)) was low (0.1% / 0.004%). No significant differences were encountered for the rates of NILM and IVa-p+ rates of MN III compared to MN II, but with MN III it was possible to determine the ASC-US, LSIL and HSIL rates and the frequency of glandular lesions in German cytology labs . About one third of the cases with invasive cancer cytology (group V, 0.02%) were endometrioid (V-e, 29%), only 12% glandular endocervical (V-g) and 51% of squamous differentiation.

Conclusion

MN II has successfully been replaced by MN III which is fully compatible with TBS. The frequency of ASC-US (group II-p) is lower than the LSIL, of ASC-H (III-p) considerably lower than HSIL rate, which indicates a good standard of quality in cytological screening diagnoses. The three tier system of squamous dysplastic lesions allows for a differentiated approach in follow-up (cytology for IIID1 and first time IIID2; colposcopy or excision biopsy for recurrent IIID2 or for IVa/b-p), and the determination of glandular or presumably glandular origin helps in the appropriate diagnostic work-up and proper therapeutic procedures for patients with these lesions.

References

Griesser H, Breinl H, Jordan B: Münchner Nomenklatur III. Gynäkologische Dysplasien werden klar zugeordnet. Dtsch Aerzteblatt 2014; 111: A640.

Griesser H, Marquardt K, Jordan B, et al.: Das Prozedere bei auffälligen Befunden. Kommentar zur Münchner Nomenklatur III. Frauenarzt 2015; 56: 10-33.

Marquardt K, Griesser H: Münchner Nomenklatur III: Befundverteilung in der Jahresstatistik. Frauenarzt 2015; 56: 108-110.

S3 LEITLINIE PRÄVENTION DES ZERVIXKARZINOMS

P. Hillemanns¹, M.W. Beckmann², C. Dannecker³, M. Fehr⁴, M. Friedrich⁵, M. Gebhardt⁶, C. Grimm⁷, T. Iftner⁸, H. Ikenberg⁹, S. Klug¹⁰, T. Löning¹¹, K. Münstedt¹², M. Pawlita¹³, K.U. Petry¹⁴, A.P.A. Schäfer¹⁵, D. Schmidt¹⁶, A. Schneider¹⁷, U. Siebert¹⁸, M. Jentschke¹⁹

¹Department of Obstetrics and Gynaecology, Hannover Medical School, Hannover (Germany), ²Department of Obstetrics and Gynaecology, University of Erlangen, Erlangen (Germany), ³Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University (LMU), Munich (Germany), ⁴Department of Obstetrics and Gynaecology, Spital Thurgau AG, Frauenfeld (Switzerland), ⁵Department of Obstetrics and Gynaecology, Helios Hospital, Krefeld (Germany), ⁶Frauenselbsthilfe nach Krebs e.V. (Germany), ⁷Department of Obstetrics and Gynaecology, University of Vienna (Austria), ⁸Department of Virology, University of Tübingen (Germany), ⁹Cytomol, Frankfurt (Germany), ¹⁰Cancer Epidemiology, University Cancer Center Dresden (Germany), ¹¹Department of Pathology, University Hospital Hamburg-Eppendorf (Germany), ¹²Department of Gynaecology, Ortenau Hospital Offenburg-Gengenbach (Germany), ¹³German Cancer Research Center, Heidelberg (Germany), ¹⁴Department of Obstetrics and Gynaecology, Wolfsburg Hospital, Wolfsburg (Germany), ¹⁵Department of Obstetrics and Gynaecology, Charite, Berlin (Germany), ¹⁶Department of Pathology, synlab MVZ Pathologie Mannheim GmbH, Mannheim (Germany), ¹⁷IZD Berlin (Germany), ¹⁸UMIT, Hall, Tirol (Austria), ¹⁹Department of Obstetrics and Gynaecology, Hannover Medical School, Hannover (Austria)

Background / Objectives

Im Zuge des nationalen Krebsplans von 2008 und des Krebsfrüherkennungs- und Registergesetzes (KFRG, 2013) wurde die Neuentwicklung einer S3 Leitlinie zur Zervixkarzinomprävention gefordert.

Methods

Mitte 2012 wurden 21 wissenschaftliche Fachgesellschaften und Berufsverbände eingeladen, Mandatsträger für die S3 Leitlinie zu bestellen. Mit finanzieller Unterstützung der Deutschen Krebshilfe und unter wissenschaftlicher Begleitung durch die AWMF und das Leitlinienprogramm Onkologie (OL) wurden in 16 Arbeitsgruppen evidenzbasierte Statements und Empfehlungen erarbeitet (Evidenzbewertung nach GRADE). Zwei unabhängige Institute (M. Arbyn, WIV-ISP, Belgium; J. Kleijnen, KSR, England) erstellten systematische Reviews für die Leitlinie.

Results

Auch in der Leitlinien-Metaanalyse zeigte sich, dass ein HPV Screening besser vor invasiven Zervixkarzinomen schützt, als ein Zytologie basiertes Screening. Deshalb soll bei Frauen ab 30 Jahren ein organisiertes Screening bevorzugt mittels alleinigem HPV Test (validierte Testverfahren und Labore) mit einem Intervall von mindestens 3 Jahren oder alternativ mittels Zytologie alle 2 Jahre erfolgen. Eine Ko-Testung aus Zytologie und HPV-Test sollte nicht erfolgen. Bei Frauen zwischen 25 und 30 Jahren sollte ein Zytologie-basiertes organisiertes Screening durchgeführt werden. Für Frauen über 65 Jahren ist der Nutzen eines organisierten Screenings nicht belegt, unabhängig davon ob dieses HPV und/oder Zytologie basiert ist. Bei Frauen über 65 mit mehrfach negativen HPV Testergebnissen soll daher über eine Beendigung der Zervixkarzinomfrüherkennung gesprochen werden. Frauen, die nicht am Screening teilnehmen, sollte die Möglichkeit zum HPV-Selbstabstrich gegeben werden.

Mangels Evidenz sollen Biomarker im primären Screening nicht eingesetzt werden.

Bei einem positiven HPV-Screeningtest sollte eine weiterführende zytologische Abklärung erfolgen, alternativ kann eine Abklärung mittels p16/Ki-67-Testung erfolgen. Bei einem positiven HPV-16/18 Testergebnis im HPV-basierten Screening sollte eine kolposkopische Abklärung erfolgen. Nach positivem zytologischem Screeningbefund sollte eine Triage mittels HPV Test durchgeführt werden, alternativ auch eine p16/Ki-67-Testung.

Schlingen- und Laserexzision unter kolposkopischer Kontrolle sollen die Methoden der Wahl für die Behandlung der hochgradigen CIN sein. Bei CIN 1 und 2 soll abgewartet und die Patientin nach 6 Monaten wieder untersucht werden. In der Nachbetreuung nach Therapie einer CIN/ ACIS soll eine kombinierte Untersuchung mit HPV-Test und Zytologie durchgeführt werden.

Conclusion

Die S3 Leitlinie wurde im ersten Quartal 2016 nach einer vierwöchigen Online Konsultationsphase auf <http://awmf.org> publiziert.

GS 03-03

WELCHER HPV TEST IST FÜR DAS SCREENING GEEIGNET?

T. Iftner

**Sektion Experimentelle Virologie, Institut für Medizinische Virologie,
Universitätsklinikum Tübingen (Germany)**

Background / Objectives

Ergebnisse verschiedener randomisiert-kontrollierter Studien (RCT) zeigen, dass HPV-Tests sensitiver als die zytologische Untersuchung sind und effektiv im Primärscreening von Frauen über 30 Jahren verwendet werden können. HPV-Tests haben eine geringere Spezifität für den Nachweis von CIN3+ Läsionen als die Zervixzytologie, sind jedoch wesentlich besser reproduzierbar. Um HPV-Tests in die primäre Gebärmutterhalskrebsfrüherkennungsuntersuchung einzuführen, müssen sie bestimmte Kriterien erfüllen, von denen einige allgemein anerkannt sind.

Methods

Conclusion

Ein HPV-Test muss in der Lage sein, alle 13 Hochrisiko-HPV-Typen zu identifizieren, die als Klasse I oder Klasse IIa Karzinogene durch die WHO (IARC) klassifiziert wurden. Die klinische Sensitivität und Spezifität eines HPV-Tests für den Nachweis von CIN2+ sollte mindestens 90% bzw. 98% der Sensitivitäten und Spezifitäten bereits etablierter und validierter HPV-Tests (z.B. HC2) sein und sollte in Proben von Frauen einer Routine-Screeningbevölkerung im Alter von 30 Jahren und älter gemessen werden. HPV-Tests können im großen Maßstab durchgeführt werden und die Verfügbarkeit von kommerziell erhältlichen Testkits ermöglicht es, qualitätsgesicherte Testergebnisse zu generieren. Weiterhin ist es notwendig, dass alle HPV-Tests, die im Primärscreening eingesetzt werden sollen, nach Herstelleranweisung und nach validierten Standard-Betriebsverfahren in akkreditierten Labors durchgeführt werden. HPV Tests müssen außerdem zu einem hohen Maß automatisierbar sein, um das Risiko von Kontaminationen oder Fehlern durch manuelle Handhabung der Proben zu vermeiden und hohe Durchsatzraten zu erreichen. Aufgrund der besonderen Bedeutung von HPV16, sollte die HPV Genotypisierung Teil des Primärscreenings sein. Das HPV-

basierte Primärscreening erfordert angemessene Triage-Optionen für das Management HPV-positiver Frauen. Diese Triage-Strategien sollten basierend auf Demonstrationsvorhaben überwacht und umgesetzt werden. Da dadurch zunehmend komplexere Screening-Algorithmen geschaffen werden, sollten HPV-Tests im Rahmen des organisierten Screenings durchgeführt werden, um eine angemessene Nachverfolgungsstrategie der positiven Testergebnisse zu gewährleisten.

GS 03-04

HPV-TESTUNG IN DER ROUTINE: KORRELATION MIT DER HISTOLOGIE

H. Ikenberg, C. Börsch, A. Bernhardt, Z. Ilona, X. Arjola

Cytomol, Laboratory for Cytology and Molecular Biology, D-60437 Frankfurt (Germany)

Background / Objectives

Kürzlich wurde die Sensitivität der high-risk-HPV-Testung für invasive Zervixkarzinome in Zweifel gezogen.¹ Daher haben wir den HPV-Status von Patientinnen mit histologisch bestätigten CIN 2 und 3 sowie Zervixkarzinomen (CIN 2+), getestet unter Routinebedingungen vor Biopsie und/oder Therapie, mit den entsprechenden Histologiebefunden verglichen.

Methods

Alle Fälle eines deutschen Routinelabors (Cytomol, Frankfurt), in denen eine histologische Untersuchung im Jahr 2013 durchgeführt wurde und Ergebnisse eines DNA-Tests auf HPV- high-risk-Typen (maximal sechs Monate früher) vorlagen, werden berichtet. Die Histologie folgte einer kolposkopisch geleiteten Biopsie, Konisation oder Hysterektomie. Alle HPV-Tests wurden nach den Vorschriften der Hersteller aus Zervixabstrichen durchgeführt, die in Abnahmegefäßen der Hersteller oder in Thinprep-Gefäßen (Hologic, Wiesbaden) vorlagen. 55.0% der Tests wurden mit dem HC2-Test (Qiagen, Hilden) und 45.0% mit dem cobas-Test (Roche Diagnostics, Mannheim) durchgeführt. Alle zytologischen Untersuchungen und HPV-Tests wurden zentral bei Cytomol, die histologischen Untersuchungen in zahlreichen regionalen pathologischen Instituten durchgeführt.

Results

In 784 von 1004 histologisch bestätigten CIN 2+ Fällen lag ein HPV-Ergebnis vor (78.1%). 737 (97.4%) der CIN 2 und 3 waren HPV-positiv und 20 (2.6%) HPV-negativ. Die Positivitätsrate unterschied sich nicht zwischen CIN 2 und 3. 25 von 27 (93%) invasiven Zervixkarzinomen, bei denen ein HPV-Ergebnis vorhanden war, waren HPV-positiv, zwei (7%) HPV-negativ. Von den insgesamt 22 HPV-negativen CIN 2+ waren 10 (45%) mit HC2 getestet und 12 (55%) mit cobas. Somit waren 2.3% der HC2- und 3.4% der cobas-Tests bei CIN 2+ negativ. Von den 15 HPV-negativen CIN 2 und 3, bei denen p16/Ki-67-Analysen durchgeführt wurden, waren 14 (93%) p16/Ki-67-positiv. Daten aus einem erweiterten Zeitraum werden berichtet.

Conclusion

Die große Mehrzahl histologisch bestätigter CIN 2 und 3 sowie invasiver Zervixkarzinome war HPV-HR-positiv wenn der Test aus Zervixabstrichen durchgeführt wurde, welche weniger als 6 Monate vor der Biopsie und/oder Therapie entnommen wurden. Die ohnehin sehr niedrige Rate HPV-negativer Befunde war noch etwas geringer mit dem HC2-Test. Ein sehr hoher Prozentsatz der HPV-negativen Fälle war positiv für den Biomarker p16/Ki-67. Diese Ergebnisse aus der Routine deuten auf eine hohe Sensitivität des high-risk-HPV-Tests, aber auch des p16/Ki-67-Nachweises für CIN 2+ und gerade auch für das invasive Zervixkarzinom hin.

References

¹Blatt AJ, Kennedy R, Luff RD, Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. *Cancer Cytopathol.* 2015 May;123(5): 282-8. doi: 10.1002/cncy.21544. Epub 2015 Apr 10.

GS 03-05

Triage von Frauen mit auffälligen Befunden - Rolle von Biomarkern?

N. Wentzensen

Division of Cancer Epidemiology and Genetics National Cancer Institute - Bethesda (United States of America)

Background / Objectives

Trotz zunehmender HPV-Impraten bleibt die Früherkennung in den nächsten Jahrzehnten weiterhin eine wichtige Komponente der Zervixkarzinom-Prävention. Die Einführung von HPV Tests im primären Screening bietet eine Alternative zum Zytologie-basierten Screening. Während die Sensitivität des HPV-Tests viel höher als die der Zytologie ist, sind die meisten HPV-Infektionen harmlos und zusätzliche Tests werden benötigt um Frauen mit Präkanzerosen zu identifizieren. Derzeit empfohlen Triage-Strategien für das primäre HPV-Screening sind die Genotypisierung für HPV16 und HPV18 und die Zytologie. Andere Alternativen, die derzeit untersucht werden und vielversprechende Ergebnisse für die Triage gezeigt haben, sind p16/Ki-67 dual stain Zytologie, sowie zelluläre und virale Methylierungstests. Aufgrund der Vielzahl von Optionen für das primäre Screening und das Management werden zunehmend Risiko-basierte Ansätze verfolgt, die es erlauben, klinisches Management unabhängig von einzelnen Testergebnissen zu definieren. Spezifische Risikoschwellen werden zum Beispiel für die Rückkehr in das primäre Screening, Triage, Überweisung zur Kolposkopie, und sofortige Behandlung definiert. Eine optimale integrierte Screening- und Triage-Strategie sollte der überwiegenden Mehrheit der Frauen versichern, dass sie ein sehr niedriges Risiko von Gebärmutterhalskrebs haben, während die Frauen mit dem höchsten Risiko zum richtigen Zeitpunkt zu Kolposkopie überwiesen werden sollten.

GS 04-01

THERAPEUTISCHE VAKZINE GEGEN HPV – WO STEHEN WIR?

A.M. Kaufmann

Charité-Universitätsmedizin Berlin (Germany)

Background / Objectives

Die Entwicklung von therapeutisch wirksamen Impfstoffen gegen humane Papillomviren (HPV) reicht länger zurück als die der präventiven Vakzine. Das Konzept, gegen immunogene Antigene eine zelluläre kurative Immunität zu induzieren war naheliegend. Das Virus führt körperfremde Antigene in die Wirtszellen ein und in >90% der Infizierten kommt es zu einer Ausheilung von Infektionen oder einer spontanen Regression von Dysplasien. Allerdings zeigt sich bereits bei Präkanzerosen eine Abnahme der Regressionswahrscheinlichkeit mit dem Schweregrad der Dysplasie und invasive Karzinome regredieren nur sehr selten spontan. Dafür wird eine Immunselektion und „immune escape“ der HPV positiven Tumorzellen verantwortlich gemacht, die nach Jahren der Auseinandersetzung und Durchsetzung gegen eine aktive Immunantwort zu immunresistenten Varianten führt.

Methods

Klinische Studien mit einer Vielzahl an Immunisierungsstrategien wurden zunächst bei fortgeschrittenen Tumorstadien, in der Regel ohne nennenswerte Erfolge, ausgetestet. Nach einer Periode der Frustration sind derzeit neue Initiativen mit innovativen Impfansätzen in klinischer Prüfung und haben Phase II Studienniveau erreicht. Hierbei kommen rekombinante Antigene, DNA- und Virusvektor-basierte Impfstoffe und auch zelluläre Proteine wie P16 als Targets zum Einsatz. Die Besonderheit in dieser 2. Welle der therapeutischen Impfstoffentwicklung ist, dass die Zielerkrankung häufig frühere Krankheitsstadien sowie bloße HPV-Infektionen sind. Dies kommt aus dem Verständnis, dass eine sekundäre Prävention in dieser Situation der Immunselektion zuvor kommt und damit erfolgversprechender ist. Weitere Neuerungen betreffen Adjuvantien und immunologische „checkpoint Inhibitoren“, die ein großes Potential für die Überwindung einer HPV-induzierten oder selektionsbedingten Immunsuppression bergen.

Conclusion

Die Persistenz bei der Entwicklung HPV-spezifischer therapeutische Impfstoffe in Akademie und Industrie verspricht zukünftig die Persistenz von HPV Infektionen und Dysplasien zu verhindern.

GS 04-04

THERAPIE DER HPV ASSOZIIERTEN ERKRANKUNGEN DER VULVA

M. Hampl

University Hospital of Duesseldorf, Department of Gynecology and obstetrics (Germany)

Background / Objectives

Eine persistierende HPV Infektion der Vulva kann Präneoplasien und Neoplasien hervorrufen. Die HPV induzierten Erkrankungen des Genitaltraktes können in low und high risk induzierte Läsionen unterteilt werden. Typische durch low risk Infektionen induzierte Veränderungen sind Genitalwarzen, high risk induzierte Erkrankungen stellen die VIN und das z.T. das Vulvakarzinom dar.

Methods

In diesem Vortrag wird die Diagnostik und Therapie dieser Läsionen dargestellt.

Results

Eine der häufigen gutartigen Veränderungen stellen die Genitalwarzen dar, die in 90% der Fälle durch eine Infektion mit den low risk HPV Viren Typ 6/11 induziert werden. Nach einer Inkubationszeit von 3-6 Monaten entstehen die Condylome im Genitalbereich, diese können spontan abheilen oder werden mit Immunmodulationen wie Imiquimod oder Veregen, cytotoxischen Substanzen wie Podophylloxylin oder TCA behandelt oder chirurgisch entfernt (Laser, Elektrotherapie oder Skalpell). Die Rezidivraten sind abhängig von der Therapieart und schwanken zwischen 10 und über 30 %. Eine Primärprävention der Erkrankung stellt die Impfung mit dem Vierfach HPV Impfstoff dar, die zu fast 100% die Entstehung von Genitalwarzen bei HPV naiven Menschen verhindert. Präneoplasien in Form von intraepithelialen Neoplasie an Vulva oder perianal entstehen in ca. 90% der Fälle durch Infektionen mit den high risk HPV- Viren 16, 18, 31 oder 33 und bergen das Risiko des Progresses zum invasiven Karzinom in sich. Die Progressionsrate einer VIN 3 liegt bei 8-10%. Die Standardtherapie ist die Entfernung in sano mittels Laser oder Excision, im off label use kann auch hier eine Therapie mit Imiquimod unter kurzfristigen Kontrollen erfolgen, da ein Progress zum Karzinom auch unter dieser Therapie auftreten kann. .

Das invasive Vulvakarzinom ist nur in ca. 40 % ein HPV induzierter Tumor, der größere Anteil dieser Karzinome entsteht auf dem Boden eines Lichen sclerosus et atrophicus oder ist altersbedingt. Die Prädisposition ist die vordere Kommissur zwischen Klitoris und Urethra (bei 60% der Frauen) Die

Therapie dieser Tumore ist chirurgisch mit in sano Excision, Hemivulvektomie oder Vulvektomie mit LK Entfernung in der Leiste oder Sentinel LNE (je nach Größe). In inoperablen Fällen oder Befall von Nachbarorganen kann eine Radiochemotherapie den Tumor zum Regress bzw. Abheilung bringen.

Conclusion

HPV induzierte Vulvaerkrankungen können primärpräventiv angegangen werden durch eine HPV Impfung junger Frauen mit dem HPV 6/11/16/18 Impfstoff oder in Zukunft mit dem nonavalenten Impfstoff

EUROGIN 2016 ABSTRACTS

Part II – Oral Communications (OC)

OC 01.....	P.2
OC 02.....	P.23
OC 03.....	P.43
OC 04.....	P.59
OC 05.....	P.85
OC 06.....	P.101
OC 07.....	P.122
OC 08.....	P.140
OC 09.....	P.163
OC 10.....	P.183
OC 11.....	P.196
OC 12.....	P.232
OC 13.....	P.269
OC 14.....	P.302
OC 15.....	P.336

OC 01-01

EUROPEAN HPV DNA TEST EXTERNAL QUALITY ASSURANCE SCHEME (EHEQAS)

P. Neophytou¹, J. Konya², R. Tachezy³, C. Kroupis⁴, M. De Koning⁵

¹Mendel Center for Biomedical Sciences, Egkomi, Nicosia (Cyprus),

²Department of Medical Microbiology, University of Debrecen Medical School (Hungary), ³Institute of Hematology and Blood Transfusion, National Reference Laboratory for Papillomaviruses, Prague (Czech republic), ⁴Attikon University General Hospital, University of Athens Medical School (Greece), ⁵DDL Diagnostic Laboratory, Rijswijk (Netherlands)

Background / Objectives

To improve the quality of laboratories in HPV detection and typing we run the program of external quality control - EHEQAS.

Methods

EHEQAS was founded in 2006 and by 2015 already 23 laboratories from 7 European countries are participating. Batches of 5-7 samples are sent from the coordinator to participants 1-2 times per year. Samples are either real patient samples (including cervical cell pellets) or prepared from international standards as standalone dilutions or mixtures with real patient samples. Samples that are not international standards are pre-tested by reference laboratories and only samples for which there is a high level of agreement between reference laboratories are used. To test for reproducibility, samples are used in duplicate in the same and in different rounds. Linearity is evaluated by different dilutions of the same sample in the same and in different rounds. Results are evaluated and consensus results are issued and announced to participants in a confidential way. Marks are awarded to participants based on defined rules that reflect the clinical value of the result (e.g. higher penalty for errors regarding types 16 and 18). Certificates of competence that reflect the performance of a laboratory during the past 4 years are issued.

Results

Until now 205 samples have been tested in 20 rounds: 57 negative, 57 single infections, 91 co-infections. 31 different types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 68, 70, 73, 81, 82, 83, 84) were detected during the period 2006-10 and the same 31 types were also fully represented during the period 2011-15. Laboratories using IVD tests made significant

errors in HPV detection and typing, depending on the skills of laboratory personnel and on whether they correctly followed manufacturer's instructions.

Conclusion

There is a gradual increase in the number of participants and in the quality of their performance. EHEQAS improves quality with the coordinating team providing feedback to participants on how to improve their methodology. EHEQAS assesses the quality of laboratories in: (a) detecting a shift in sensitivity and specificity in time. (b) HPV typing (high- or low-resolution). Successful participation in EHEQAS is extremely helpful to high-quality HPV labs that also verify or validate their methods: success in an EQA is a prerequisite for granting ISO15189 accreditation.

OC 01-02

VALIDATION OF HPV DNA ARRAY GENOTYPING ASSAY WITH CERVICAL CANCER SAMPLES

A.P. Pesic¹, A.K. Krings¹, M.H. Hempel², A.M. Mathewos³, J.K. Komp⁴, D.H. Höfler⁵, D.H. Holzinger⁵, M.P. Pawlita⁵, E.J.K. Kantelhardt⁴, A.M.K. Kaufmann¹

¹Gynäkologische Tumorimmunologie, Charité Universitätsmedizin Berlin (Germany), ²AID/GenID Diagnostika, Strassberg (Germany), ³Radiotherapy Center, Addis Ababa University Hospital, Addis Ababa (Ethiopia), ⁴Institute for Medical Epidemiology, Biostatistics and Informatics, Martin Luther-University Halle, Germany (Germany), ⁵German Cancer Research Center (DKFZ), Heidelberg (Germany)

Background / Objectives

The purpose of this study was to evaluate the performance of HPV DNA Array for full genotyping of 18 high-risk (16,18,26,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82) and 11 low-risk HPV types (6,11,40,42,44,54,67,69,70,85,97), by using 110 histology confirmed, HPV-DNA positive, cervical cancer samples pre-genotyped by multiplex Luminex-based hybridization assay following BS-GP5+/6+ PCR (BS-GP5+/6+ MPG).

Methods

HPV DNA Array is an E1-based PCR assay. Multiplex PCR is used for amplification of E1 gene sequences of 180bp length. The amplicons are detected and genotyped by reverse hybridization to immobilized DNA probes spotted as triplets in single 96 well-plate wells and read by AID EliSpot reader system and AiDOT software. Assay performance was assessed with 110 cervical cancer samples collected with Delphi screener cervico-vaginal lavage and stored in PreserveCyt. Ethical approval was given by the Institutional Review Board of Addis Ababa University, Ethiopia. Patients were recruited after giving informed consent at Tikur Anbessa teaching hospital, Addis Ababa. HPV detection accuracy was established by comparing HPV DNA Array to BS-GP5+/6+ MPG, as gold standard. BS-GP5+/6+ MPG is L1-based genotyping test. Multiplex PCR is used for amplification of L1 gene sequences of 150bp length.

Results

Concordance of HPV genotype related positivity identified by the two assays was 90%. We observed a complete genotype match in 64.5% (71/110), ≥ 1 HPV genotype

match in 25.5% (28/110), with discordant BS-GP5+/6+ MPG positivity/HPV DNA Array negativity in 7.3% (8/110) and concordant HPV positivity with discrepant types in 2.7% (3/110). The HPV DNA Array showed sensitivity for overall HPV detection of 92.5% with sensitivity for detection of HPV-16: 93% (66/71), HPV-18: 83% (5/6), and HPV-45: 67% (8/12), compared to BS-GP5+/6+ MPG.

Conclusion

In most or all HPV DNA Array false negative HPV-16 (4/5 cases) and HPV-45 samples (4/4 cases), we observed a low HPV copy number in BS-GP5+/6+ MPG. Also in 7 out of 8 discordant samples (BS-GP5+/6+ MPG positive/HPV DNA Array negative), low MPG-Luminex signals were found, arguing for insufficient sample quality.

Overall, HPV DNA Array showed good sensitivity for HPV detection, and due to its technical simplicity and high-throughput potential, this method may be suitable for routine full genotyping in HPV screening.

OC 01-03

INTER-LABORATORY REPRODUCIBILITY OF THE COBAS 4800 HPV TEST IN CERVICAL CANCER SCREENING IN NORWAY

B. Engesæter¹, B. Van Diermen Hidle², M. Hansen³, P. Moltu², K. Mangseth Staby⁴, S. Borchgrevink-Pedersen⁵, O.K. Vintermyr⁴, S. Lönnberg¹, M. Nygård¹, E.A. Janssen², P. Castle⁶, I.K. Christiansen³

¹Cancer Registry of Norway, Oslo (Norway), ²Department of Pathology, Stavanger University Hospital, Stavanger (Norway), ³Department of Microbiology and Infection Control, Akershus University Hospital, Lørenskog (Norway), ⁴Department of Pathology, Haukeland University Hospital, Bergen (Norway), ⁵Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim University Hospital, Trondheim (Norway), ⁶Albert Einstein College of Medicine, Bronx, NY (United States of America)

Background / Objectives

Human papillomavirus (HPV) testing by the Cobas 4800 HPV test as primary screening for cervical cancer is currently being implemented in Norway in a randomized controlled fashion. To confirm satisfactory HPV test reliability, an evaluation of inter-laboratory reproducibility between the laboratories involved in the implementation was initiated.

Methods

The Cobas HPV test simultaneously detects 14 high-risk types, including individual genotype of HPV16 and HPV18. In addition to the three laboratories involved in the implementation, the Norwegian HPV reference laboratory was included as a fourth comparative laboratory. A stratified sample of 500 cervical LBC samples was used in the evaluation, with an aim towards a high-risk HPV positivity of ~25%. Samples were collected at one laboratory, anonymized, aliquoted, and distributed to the other laboratories.

Results

Considering all specimens, there was a 95.6% overall agreement, an 86.3% positive agreement, and a kappa value of 0.94 (95% CI 0.92-0.97). For negative cytology specimens, there was a 95.8% overall agreement, a 67.4% positive agreement, and a kappa value of 0.88 (95% CI 0.80-0.93). For abnormal cytology specimens, there was a 95.8% overall agreement, a 95.5% positive agreement, and a kappa value of

0.86 (95% CI 0.71-0.97). Ranking the test results according to cancer risk, there was a 94.4% overall agreement, an 82.5% positive agreement, and a kappa value of 0.94 (95% CI 0.91-0.96).

Conclusion

The study showed a high inter-laboratory reproducibility of HPV testing by the Cobas 4800 HPV test, implying satisfactory user performance and reliability in the laboratories involved in the implementation project.

OC 01-04

Validation of intra- and inter-laboratory reproducibility of the Xpert HPV assay according to the international guidelines for cervical cancer screening

D. Vanden Broeck¹, A. Akbari¹, I. Benoy¹, J. Bogers¹, E. Padalko², M. Arbyn³

¹Department of Cytopathology and Molecular Diagnostics, Algemeen Medisch Laboratorium, Antwerpen, Belgium (Belgium), ²Department of Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium (Belgium), ³Unit of Cancer Epidemiology & Belgian Cancer Centre, Scientific Institute of Public Health, Brussels, Belgium (Belgium)

Background / Objectives

Validated high-risk human papillomavirus (hrHPV) assays are more effective than cytology in cervical cancer screening however the current assays take several hours to complete. The Cepheid GeneXpert HPV assay (Xpert HPV) is a novel, non-batch real-time PCR assay capable of detecting 14 hrHPV types in 1 hour.

Methods

One of the three validation criteria for the use of a novel hrHPV DNA assay in cervical cancer screening pertains to reproducibility. This study aims to determine whether the lower bound of 95% confidence interval of the intra- and inter-laboratory agreement of the Xpert HPV exceeds 87% with kappa ≥ 0.5 .

Results

A panel of 510 cervical cell samples from women attending screening, composed in the AML laboratory (Antwerp, Belgium) were retested twice with Xpert HPV within AML and subsequently retested in the virology laboratory of University Hospital Ghent.

Results: Xpert HPV showed high intra-laboratory reproducibility with an overall agreement of hrHPV positivity of 96.9% (95% CI, 95.0 to 98.2%) with kappa = 0.925 (95% CI 0.888-0.961). Inter-laboratory testing showed an agreement of 97.8% (95% CI, 96.2 to 98.9%) with kappa = 0.948 (95% CI 0.917-0.978).

Conclusion

Retesting for presence of hrHPV DNA in cervical cell samples with the Xpert HPV shows a high level of concordance. The assay fulfills the international reproducibility criterion for use in cervical cancer screening.

OC 01-05

HPV Test of Cure (TOC) for treated CIN in 14,000 women - An analysis of 3½ years' national data from Scotland

T. Palmer¹, K. Cuschieri², C. Moore², L. Smart³, M. Cruickshank⁴, C. Busby-Earle⁴, K. Kavanagh⁵

¹Department of Pathology, University of Edinburgh, Edinburgh, Scotland. (United kingdom), ²Scottish HPV Reference Laboratory, Edinburgh Royal Infirmary, Edinburgh, Scotland. (United kingdom), ³Department of Pathology, Aberdeen Royal Infirmary, Aberdeen, Scotland (United kingdom), ⁴Department of Gynaecology, Aberdeen Royal Infirmary, Aberdeen, Scotland (United kingdom), ⁵Department of Mathematics and Statistics, University of Strathclyde, Glasgow, Scotland (United kingdom)

Background / Objectives

Scotland introduced hr-HPV testing to the follow-up of women treated at colposcopy for CIN in April 2012. Women who test hr-HPV negative and cytology negative or borderline/ASCUS are deemed 'cured' and return to routine recall in 3 years. Women who test positive for hr-HPV and/or cytology (\geq LG dyskaryosis) are referred for colposcopy. Women with a negative colposcopy examination remain under annual cytology surveillance for 2 (CIN1) or 5 years (CIN2/3). Routine cytology has now been received from many women returned to routine recall.

Methods

Data on hr-HPV and cytology test dates and results, date and outcome of follow-up colposcopy and follow-up cytology tests were obtained from the national screening database. The sensitivity, specificity, PPV and NPV of cytology alone, hr-HPV testing alone and both (as a cotest) for recurrent/residual CIN at 6 months has been calculated. The prevalence of abnormal cytology in women managed by TOC was compared to the routine screening population.

Results

By 30 November 2015, 14,156 women had undergone TOC, of whom 10,579 were returned to routine recall and 3577 were referred for colposcopy. 1,339 women given routine recall have had more than 37 months of follow-up, of whom 801 have had subsequent cytology. Only 1 case (0.12%) of HG cytology was found in this population. 852 of the 9240 women (9.22%) returned to routine recall with less than 37 months follow-up have been rescreened before their due date.

213 women (5.95% of referrals) were referred for follow-up colposcopy with positive cytology only, 2,553 (71.77%) with a positive hr-HPV test only and 811 (22.67%) women had both tests positive. Sensitivity, specificity, PPV and NPV for detection of recurrent/residual CIN2+ at 6 months following treatment are shown in the table. 45 out of 223 cases of CIN2+ were positive with only one test (9 cytology only, 36 hr-HPV only). 3157 of women referred (88.26%) had negative follow-up colposcopy, of whom 1958 have returned for cytological follow-up. 66 additional cases of HG dyskaryosis have been identified and 205 cases of LG dyskaryosis in their period of follow-up of up to 36 months.

	Cytology performance	Co-test performance	hr-HPV performance
Sensitivity	83.86%	79.82%	95.96%
Specificity	75.19%	81.48%	6.05%
PPV	18.42%	22.14%	6.39%
NPV	98.59%	98.34%	95.73%

Conclusion

Our results support the use of both cytology and hr-HPV testing in the follow-up of treated CIN. The selective use of either test will miss some cases of CIN2+ at six months, and cytological follow-up of negative colposcopies is important. Routine recall of women 'cured' is safe.

OC 01-06

VALIDITY TESTING OF CERVICAL SAMPLES INTENDED FOR ONCOPROTEIN-BASED CERVICAL CANCER SCREENING

M. Thiessen ¹, I. Koch ¹, S. Mcnamara ¹, A.M. Kaufmann ², F. Rupp ², I. Hagemann ³, E. Boschetti ², I. Drechsler ², A.S. Vetter ¹, M. Fleischhauer ¹, M. Kellner ¹, S. Fehrmann ¹, C. Reichhuber ¹, K. Chatzistamatiou ⁴, T. Agorastos ⁴, E. Soutschek ¹, O. Böcher ¹

¹Mikrogen GmbH, Neuried (Germany), ²Clinic for Gynaecology, Charité-Universitätsmedizin Berlin (Germany), ³abts+partner, Kronshagen (Germany), ⁴Depts of Obstetrics and Gynecology Hippokrateio Hospital, Aristotle University of Thessaloniki (Greece)

Background / Objectives

Cervical cancer is one of the leading causes of cancer morbidity and mortality in women, with more than 98% related to a human papilloma virus (HPV) infection origin. HPV is known to infect basal keratinocytes found within the cervical transformation zone.

The stratified squamous epithelium of the cervix consists of multiple layers of cells. In a typical cervical sampling, it cannot be ruled out that only the most superficial layers are scraped away for analysis.

Promising new HPV tests based on the detection of viral oncoproteins of HPV high risk types are in the pipeline, but their diagnostic capabilities may be limited without a way to assess specimen validity. Hence, there is a dire need to reduce false negative results of these tests due to unreliable sampling.

Here we describe a new Sandwich ELISA that captures specific intermediate filament proteins (cytokeratins 5, 8 and 18) from potentially target cells located within or originating from the cervical transformation zone as a means of normalizing cervical specimens.

Methods

Cervical samples were analyzed for presence of HPV and carefully characterized via microscopy for content of cellular material and cell morphology. All samples were tested in both a newly developed keratin 5/8/18 ELISA and a pan-keratin control ELISA.

Cervical samples collected from patients participating in studies of the PIPAVIR program were used to test the feasibility of the 5/8/18 ELISA in a screening population and in patients referred to colposcopy showing various stages of cervical intraepithelial neoplasia (CIN).

Suitable for measurement of Keratin in both ELISA systems are liquid-based cytological samples (ThinPrep) as well as frozen samples.

Results

The ELISA was successfully validated with cell lysates of different cell lines of cervical origin.

The proof of concept was shown by measurement of well characterized clinical samples: with samples containing cells of the cervical transformation zone which were likely to be found and with samples showing only intermediate and/or superficial cells for demonstrating the likely absence of keratins 8, 18, and 5 in differentiated squamous cells.

In HPV positive samples of all stages of CIN, Keratin 5, 8, 18 could be detected with a similar signal distribution when compared to normal HPV positive samples.

Conclusion

Our results demonstrate the presence and detectability by ELISA of keratins 5, 8, and 18 in parabasal, squamous metaplastic, and endocervical cells, while simultaneously suggesting their absence in differentiated squamous cells. We also validate with ELISA the expression of these keratins in individuals with HPV-induced dysplasia.

References

Smedts et al, Am J Pathol. 1992 Aug;141(2):497-511.

OC 01-07

PERFORMANCE EVALUATION OF PAPILOPLEX(TM) HR-HPV KIT – A NOVEL MULTIPLEXING ASSAY FOR GENOTYPING ALL 14 HR HPV TYPES IN A SINGLE CLOSED TUBE REAL-TIME PCR REACTION

R. Bhatia ¹, M. Moreau ², E. Boland ², I. Serrano ¹, C. Graham ³, D. Kapadia ², G. Fu ², K. Cuschieri ⁴

¹Human Papillomavirus Group, University of Edinburgh, Edinburgh, UK (United kingdom), ²GeneFirst Ltd, Culham, Oxfordshire, UK (United kingdom), ³Epidemiology and Statistics Core, Edinburgh Clinical Research Facilities, Edinburgh (United kingdom), ⁴HPV Reference Laboratory, Royal Infirmary of Edinburgh, Edinburgh, Scotland, UK (United kingdom)

Background / Objectives

Multiplex Probe Amplification (MPA) technology dramatically expands multiplexing ability of a real-time PCR reaction. The technology allows detection of multiple targets in a single fluorescence channel using a mixture of hydrolysis based probes, partially complementary oligonucleotides and their melting curve analysis. The MPA technology was used to develop a quick, easy-to-use, sensitive and affordable genotyping assay that detects all 14 HR-HPV types in a single reaction – Papilloplex™ HR-HPV test.

In the present study, we carried out a comparative analysis of the performance of Papilloplex™ HR-HPV test with four well established assays on a panel of liquid based cytology (LBC) samples. Analytical specificity of the assay was also interrogated using the WHO HPV LabNet proficiency panel (2015).

Methods

The Papilloplex™ HR-HPV test was used to test 500 disease enriched cervical LBC samples obtained from the Scottish HPV Archive, Edinburgh with known concurrent pathology results. Samples were also tested by the Abbott rT HPV assay, the Qiagen Hybrid Capture 2 Assay, the Diamex Optiplex HPV Genotyping kit and Roche Linear Array HPV Genotyping test. Concordance between the comparator assays vs Papilloplex™ was performed using binomial test and McNemars test of proportions. As the samples were enriched for CIN2+ the applicability of clinical performance measures to screening settings are limited however these were assessed in terms of sensitivity and specificity for underlying CIN2+.

Results

The Papilloplex™ assay was able to detect and genotype HPV HR types 16, 18, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a and 68b in a single reaction. The limit of detection for the assay was 5 genome copy numbers for HPV 16 and 18 in the WHO LabNet samples with 100% accuracy for genotyping. The overall proportional agreement of Papilloplex™ was high with Abbott rtHPV assay [95% (CI: 93 to 97%)], HC2 [90% (CI: 87-93%)], Linear Array [96% (94. 96%)] and Optiplex [94% (92, 96%)]. Type specific concordance was also high with all four assays.

Conclusion

These data indicate that the analytical performance of Papilloplex™ HR-HPV assay is comparable to established HPV assays at the level of generic HR-HPV detection and at the type specific level. The assay shows potential promise for both disease management and epidemiological applications. Further data on the clinical performance of the assay will be presented.

OC 01-08

OPTIMIZING POINT-OF-CARE HPV TESTING FOR CERVICAL CANCER PREVENTION IN SOUTH AFRICA

L. Kuhn¹, R. Saidu², R. Boa², A. Tergas¹, C. Svanholm-Barrie³, J. Moodley², T. Wright¹, D. Persing⁴, S. Campbell⁴, L. Denny²

¹Columbia University (United States of America), ²University of Cape Town (South africa), ³Cepheid (Sweden), ⁴Cepheid (United States of America)

Background / Objectives

Single visit approaches could revolutionize cervical cancer screening improving cancer prevention in low-resource, high-burden settings. In single visit approaches, treatment (usually cryotherapy) is provided based on a positive screening test. HPV testing is known to be the most sensitive test, but true point-of-care (POC) HPV tests, which are necessary to implement this approach, have not been available until now. Some have raised concerns that specificity of HPV testing may be too low, e.g. in HIVpositive women. Here we evaluate a new POC HPV test, including optimizing it for screen-and-treat.

Methods

At a colposcopy clinic and a primary care site in Cape Town, South Africa, 213 HIVnegative and 336 HIVpositive women, 30-65 years, were recruited. All women had a cervical sample collected that was tested by the Cepheid POC HPV test (detects 14 high-risk HPV types in 5 channels: HPV16, HPV18,45, HPV31,33,35,52,58, HPV51,59, HPV39,56,66,68) yielding a result within an hour. All women underwent colposcopy with histological sampling. Cervical intraepithelial neoplasia grade 2, 3 and cancer (CIN2+) and within normal limits were diagnosed based on consensus pathology review. Using logistic regression, we evaluated whether the clinical utility of the assay could be improved through reclassification of what was considered screen-positive based on HPV types and “viral load” estimated by cycle threshold (CT) values.

Results

In HIVnegative women at the primary care site, 13.1% were positive for any of the 14 high risk types, biopsy-confirmed prevalence of CIN2+ was 4.0% and specificity was 89.2%. Specificity could be improved to 93.0% with no loss in sensitivity (88% for detection of CIN2+) if only HPV16,18,45,31,33,35,52,58 were tested for. In HIVpositive women, 47% were positive for any high risk HPV and prevalence of

CIN2+ was 15.3%. Specificity improved to 71.6% with little loss in sensitivity (92.6% for CIN2+) testing only for HPV16,18,45,31,33,35,52,58. Combination of testing for a restricted number of genotypes and limiting CT values allowed specificity to be improved to 84.7% at a sensitivity of 85%; or specificity to 78% at a sensitivity of 90%.

Conclusion

For HIV-negative women, existing cutoffs on the HPV test, esp if restricted to some high risk types, make it an excellent test for screen-and-treat in South Africa. Given high sensitivity and specificity, even if treatment is based purely on the screening test result, number of women treated unnecessarily is small. For HIV-positive women, testing for only some genotypes and limiting the CT values allows for excellent sensitivity to be retained while attaining specificities that are adequate for single visit, screen-and-treat.

OC 01-09

A NEW ELISA-BASED TOOL FOR DETECTION OF HIGH-RISK HPV E7 PROTEINS IN CERVICAL SAMPLES

I. Koch¹, **A.S. Vetter**¹, **M. Thiessen**¹, **M. Fleischhauer**¹, **M. Kellner**¹, **H. Pfister**¹, **S. Fehrmann**¹, **C. Reichhuber**¹, **E. Boschetti**², **A. Pesic**², **I. Hagemann**³, **A.M. Kaufmann**², **K. Chatzistamatiou**⁴, **T. Agorastos**⁴, **P. Jansen-Dürr**⁵, **E. Soutschek**¹, **O. Böcher**¹

¹Mikrogen GmbH, Neuried (Germany), ²Clinic for Gynaecology, Charité-Universitätsmedizin Berlin (Germany), ³abts+partner, Kronshagen (Germany), ⁴Depts of Obstetrics and Gynecology Hippokrateio Hospital, Aristotle University of Thessaloniki (Greece), ⁵Institute for Biomedical Aging Research Innsbruck Austria AND Tyrolean Cancer Research Institute, Leopold-Franzens-Universität Innsbruck, Innsbruck (Austria)

Background / Objectives

Persistent infection with high-risk human papillomavirus (hrHPV) types is a prerequisite for development of cervical dysplasia and cancer. During progression, deregulation and overexpression of viral proteins E6 and E7 occur, leading to loss of cell cycle control and neoplastic transformation. Current cervical cancer screening methods rely on cytological analyses compromised by frequent false-negative results and thus low sensitivity. HPV DNA-based tests pick up frequently infections without underlying disease leading to a low specificity. A more effective and reliable screening approach may involve exploitation of the oncoproteins E6 and E7 for specific detection of cervical dysplasia.

Methods

RabMabs with high specificity and sensitivity against the E7 protein of different hrHPV types were generated and a hrHPV E7 sandwich ELISA – *recomWell* HPV 16/18/45 - was developed for detection of the three hrHPV types with highest carcinogenicity (HPV 16, 18, and 45).

Cervical samples were collected to test the feasibility of *recomWell* HPV 16/18/45 in normal patients as well as in patients showing various stages of cervical intraepithelial neoplasia (CIN). Suitable for measurement of E7 protein are liquid-based cytological samples in PreserveCyte (ThinPrep).

Results

Validation of *recomWell* HPV 16/18/45 with recombinant proteins of 12 hrHPV types showed specific detection with minimal cross-reaction. Validation with cell lysates of cervical cancer cell lines CaSki (HPV16+), HeLa (HPV18+), and MS751 (HPV45+) detected the E7 protein in the background of other cellular proteins. As part of the technical validation, the detection limit for HPV positive cell lines was determined with at least 1250 cells for CaSki and MS751 and 500 cells for HeLa and the inter- and intra-assay variance was calculated.

The proof of concept was shown by measurement of well characterized clinical samples collected in PreserveCyte. Furthermore, sensitivity, specificity, and positive and negative predictive value (PPV/NPV) were calculated with the clinical samples.

Conclusion

E7 detection of hrHPV by ELISA is a feasible method to detect hrHPV infection and dysplasia. Sensitivity of E7 detection seems high enough as shown by testing of cell lines and clinical samples. Clinical sensitivity and specificity is under investigation.

As there is evidence to suggest that E7 expression is up-regulated in high-grade dysplasia, the *recomWell* HPV 16/18/45 may have a high potential for detection of dysplasia with a higher specificity for disease than HPV DNA or RNA-based tests and could be a means of molecular triage in reflex testing of hrHPV positive screening results with HPV 16/18 genotyping.

OC 01-10

Genome analysis of high risk HPV integration using molecular combing in cervical lesions: the IDAHO study

C. Clavel¹, **O. Graesslin**², **S. Douvier**³, **P. Judlin**⁴, **J.P. Bory**², **J.J. Baldauf**⁵, **J.L. Brun**⁶, **J. Leveque**⁷, **F. Lecuru**⁸, **E. Daraï**⁹, **S. Barradeau**¹⁰, **S. Bouchilloux**¹⁰, **M. Benchetrit**¹¹, **V. Dalstein**¹, **F. Lemée**¹⁰, **C. Barbe**¹², **A. Jacquet**¹⁰

¹CHU Reims, Laboratoire Biopathologie/INSERM UMR-S 903 (France), ²CHU Reims, Service de Gynécologie-Obstétrique (France), ³CHU Dijon, Service de Gynécologie-Obstétrique (France), ⁴CHU Nancy, Service de Gynécologie-Obstétrique (France), ⁵CHU Strasbourg, Service de Gynécologie-Obstétrique (France), ⁶CHU Bordeaux, Service de Gynécologie-Obstétrique (France), ⁷CHU Rennes, Service de Gynécologie-Obstétrique (France), ⁸AP-HP HEGP Paris, Service de Gynécologie-Obstétrique (France), ⁹AP-HP Tenon Paris, Service de Gynécologie-Obstétrique (France), ¹⁰Genomic Vision, Bagneux, France (France), ¹¹Laboratoire DIAG, Nice, France (France), ¹²CHU Reims, Pôle Recherche et Santé Publique, Unité d'Aide Méthodologique (France)

Background / Objectives

High-risk human papillomavirus (HR-HPV) are involved in cervical cancer development. Integration of HR-HPV DNA in cellular genomes is considered as a major event in cervical cancer development. Several techniques have been used to evaluate viral integration but most of them give an imperfect reflect of HPV physical status. Molecular Combing is a powerful innovative technology which allows direct and high-resolution visualization of HR-HPV genome integration pattern.

The aim of the IDAHO study is to evaluate the integration of 5 HR-HPV (16/18/31/33/45) by Molecular Combing as a biomarker of the severity and/or of the progression of cervical lesions.

Methods

The IDAHO prospective multicentric study will enroll 3,500 women aged 25-65 in 8 French University Hospitals, referred to colposcopy after an abnormal Pap smear. The first patient has been included in December 2015. The study will be divided into two phases: (1) transversal phase; at first visit, a colposcopy +/- biopsy will be performed, as well as a Pap smear for HPV genotyping and Molecular Combing; HPV integration status will be associated to colposcopy results and histological grades; (2) longitudinal phase; women with HPV positive and low-grade histological lesions will be followed-up by cytology at 6, 18 and 30 months, and by colposcopy +/-

histology at 12, 24 and 36 months. A Pap smear taken at 12, 24 and 36 months will allow to perform HPV genotyping and Molecular Combing. HPV integration status will be associated to the evolution of the lesion / infection. HPV genotyping (Innolipa) and Molecular Combing will be performed in central labs. All histological data will be reviewed by a central reading.

Conclusion

The IDAHO study will evaluate the diagnostic and prognostic values of HR-HPV integration status detected by Molecular Combing and could lead to identify a biomarker that can specifically differentiate between women with a high risk of developing cervical precancerous lesions or cancer and who therefore require treatment, from women with a low risk who require appropriate monitoring. Molecular Combing technology will be presented, as well as the detailed design of the IDAHO study.

OC 01-11

Combined Cytology, HPV E6, E7 mRNA, and Cell Cycle in an Automated, High Throughput Image Cytometer

B. Patterson¹, **A. Chargin**²

¹IncellDx, Inc. (United states minor outlying islands), ²IncellDx, Inc. (United States of America)

Background / Objectives

Cervical cancer screening is split between molecular approaches and combined cytology and molecular analysis. Here, we present a non-slide based technology to assess cytologic parameters and molecular biomarkers of cervical cancer in a single, high-throughput assay.

Methods

Using the IncellDx HPV OncoTect 3Dx assay performed on a FlowSight Image Cytometer (Merck Millipore Sigma), we combined the standard cervical cytology parameters of nuclear-to-cytoplasmic ratio (N/C ratio) and nuclear area with E6, E7 mRNA and the cell cycle parameters of post G0-G1% and aneuploidy to determine the stage of cytologic abnormality.

Results

The combination of directly measured nuclear-to-cytoplasmic ratio (N/C ratio) and E6, E7 mRNA defined with 97% accuracy the cytology result on 200 liquid-based cytology samples (NILM, LSIL, and HSIL). Of the six discrepant samples, four HSIL samples were downgraded to LSIL and two LSIL samples were more likely to be high grade based on marker expression and proliferation rate as determined by post G0-G1%.

Conclusion

Despite regulatory approval of HPV DNA as a primary screening method, the move away from cytology in the screening algorithm has been slow. A method that combines both molecular methods and cytologic parameters in a highly automated system that does not require slides would be desirable. Here, we present an approach that combines direct measurement of cytologic parameters, molecular analysis, and cell cycle with extremely high accuracy for staging cervical disease.

OC 02-01

DIFFERENCES IN MORTALITY RATE BETWEEN SCREEN-DETECTED AND CLINICALLY DETECTED INVASIVE CERVICAL CANCER IN THE NETHERLANDS

E. Jansen¹, K. Rozemeijer¹, S. Naber¹, S. Matthijsse¹, H. Van Agt¹, L. Overbeek², A. Siebers², M. Van Ballegooijen¹, I. De Kok¹

¹Erasmus MC, University Medical Center Rotterdam, Department of Public Health, Rotterdam (Netherlands), ²PALGA, the Nationwide network and registry of histo- and cytopathology in the Netherlands, Houten (Netherlands)

Background / Objectives

Outside the Netherlands, better survival rates have been reported for women with screen-detected invasive cervical cancer (ICC) than for women with clinically detected ICC. We aimed to explore whether ICCs detected within the Dutch screening program also led to a better survival than clinically diagnosed cancers.

Methods

For the first time in the Netherlands, individual screening history data from women with ICC from the nationwide registry of histo- and cytopathology (PALGA) have been linked to mortality data from the National Cancer Registry (NKR). Only women eligible for screening (i.e. aged 29-64 years) were included in the analyses. Differences in mortality between women with screen-detected ICC and women with clinically detected ICC have been analyzed using a Cox regression. The hazard ratio (HR) for ICC mortality was adjusted for age group (29-43, 44-64 years), cancer stage (FIGO 1A, 1B, 2+), and morphology (adeno, squamous, other). A follow-up time of 60 months has been used to cover for lead time bias.

Results

The screening and clinically detected group consisted of 1,295 and 1,115 women, respectively. Within the first 60 months, 57 women (4.4%) of the screening group and 195 women (17.5%) of the clinically detected group died. This corresponded with a HR of 0.24 (95% CI: 0.18-0.32). After adjusting for age, cancer stage, and morphology, a HR of 0.44 (95% CI: 0.32-0.60) was found. For women diagnosed with FIGO 1A and FIGO 1B, the HR was 0.23 (95% CI: 0.08-0.70) and 0.48 (95% CI: 0.30-0.76), respectively.

Conclusion

Women with screen-detected ICC have a significantly lower mortality rate than those with clinically detected ICC, after adjusting for age, cancer stage, and morphology. This lower mortality rate is probably due to a “within stage shift” and difference in prognosis (i.e. the distribution of slow growing tumors is probably higher within screen versus clinically detected ICCs). Still, when evaluating the effect of screening on population health, we need to be aware of these lower mortality rates in screened individuals.

OC 02-02

SOCIETAL COST OF HUMAN PAPILLOMAVIRUS RELATED CANCER IN SWEDEN 2006

E. Östensson¹, **C. Borgfeldt**², **C. Asciutto**², **J. Wennerberg**³, **L. Greiff**³,
M. Silfverschiöld³, **M.L. Lydrup**⁴, **U. Håkansson**⁵, **S. Andersson**⁶, **P.
Sparén**⁷

¹Department of Medical Epidemiology and Biostatistics, Karolinska Institutet and Department of of Women's and Children's Health, Karolinska Institutet, Stockholm, (Sweden), ²Department of Obstetrics & Gynecology, Skanes University Hospital, Lund (Sweden), ³Department of ORL, Head & Neck Surgery, Skanes University Hospital, Lund (Sweden), ⁴Department of Experimental Medical Science, Lund University, Lund (Sweden), ⁵Department of Urology, Skanes University Hospital, Malmo, Sweden (Sweden), ⁶Department of of Women's and Children's Health, Karolinska Institutet, Stockholm (Sweden), ⁷Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Background: Human papillomavirus (HPV) genotypes 16 and 18 are established as the cause of several clinically significant conditions. However, there are yet no published studies estimating the societal costs of HPV related diseases for both genders in Sweden. **Objectives:** This study aims to estimate the total societal cost associated with cervical dysplasia, invasive cervical cancer, cancer of the vulva, vagina, anus penis and head and neck (oropharyngeal cancer) attributable to HPV 16 and 18 in 2006, one year before introduction of HPV vaccine in Sweden.

Methods

Methods: This is an ongoing investigative report for which we used a prevalence based cost-of-illness (COI) approach. National registry diagnosis-specific data were used to estimate disease prevalence, resource utilization for inpatient and outpatient care and related direct and indirect costs.

Results

Results: Preliminary results show that the total societal cost of HPV related diseases was estimated at €113 million (€12 per inhabitant), of which €41 million (€4/inhabitant) was health care services costs and €73 million (€8/inhabitant) was loss of productivity due to morbidity (early retirement and sick leave) and mortality

(premature death). Costs due to premature death amounted to approximately €40 million (35% of the total cost). Resource utilization in inpatient care amounted to 24% of the total cost. Cervical cancer was the most costly cancer diagnoses with a total societal cost of €60 million. For cancer diagnosis affecting both genders, the societal cost of head and neck cancer was the most costly diagnosis with €19 million of which 70% of the total cost was linked to males.

Conclusion

Conclusion: The annual societal cost for HPV related cancer diseases is substantial and constitutes a major public health issue. The societal cost of oropharyngeal cancer carried by males were found to be cost drivers relevant for future decisions about including males into the national HPV school vaccination program.

OC 02-03

STANDARDIZED CASE-CONTROL AUDITS OF CERVICAL CANCER CASES FOR INCREMENTAL OPTIMIZATION OF SCREENING: AN EXAMPLE FROM SWEDEN

B. Andrae¹, K.M. Elfström², P. Pär Sparén¹

¹Dept of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden), ²Dept of Laboratory Medicine, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Real-life effectiveness of screening programs can be very different from theoretical gains. Nationwide case-control audits with standard operating procedures and HPV data can be used for repeat evaluations over time, including whether implemented improvements work as expected and provide evidence for incremental improvements of programs and prioritization of QA efforts. In countries with efficient screening programs, most cervical cancers have been prevented (1). The residual morbidity represents cancer cases still encountered. To study the effect of the different elements in the screening process, and if there is need for new recommendations and guidelines, the analyses needed require linkage to registers and comparison with control subjects from the population and should be performed in a research setting using validated data (2).

Methods

In the updated Swedish national case control audit all 4273 cxcx cases from 2002 to 2011 were clinically and histopathologically verified, and age-matched to 30 population-based controls in a nested case-control design. Complete screening histories for cases and controls were reviewed for a 10-year period using Swedish National Cervical Screening Registry (NKCx). Incidence rate ratios (IRRs), with 95% confidence intervals (CI), of cervical cancer according to screening history were estimated using conditional logistic regression models

Results

Women with two negative Pap smear within two recommended screening intervals had a seven times lower risk of squamous cell carcinoma than women who had not been screened (IRR = 0.14, CI = 0.13 to 0.16). Risk for adenoca (IRR = 0.40, CI = 0.32 to 0.49) was also reduced. Risk was particularly reduced for advanced cancers

(IRR = 0.10, CI = 0.09 to 0.13). For older women with repeat normal smears to and including age 60, risk for advanced cancer remains low for decades (IRR = 0.17, CI = 0.13 to 0.22). 12 percent of cases occurred in women with repeat normal smears. A majority of these were screen-detected micro-invasive cancers with a high chance of cure by conservative means of treatment (3)

Conclusion

The questions addressed by audits should be relevant and helpful for the responsible actors at the different levels of the screening program. They should address the organization to optimize participation, testing quality, screening test methods, triage, referrals, assessment, treatment, and follow up, as well as the authors of new guidelines. Issues regarding validity of the data should be addressed and evaluations should be comparable between countries. The standardized audit protocol will be repeated regularly to monitor effectiveness of changes, including upcoming switch to HPV-based primary screening

References

1. Vaccarella S. et al BJC 2014; 2. Andrae B. et al JNCI 2008; 3. Andrae B. et al BMJ 2012

OC 02-04

DISTRIBUTION OF CERVICAL SQUAMOUS CELL- AND ADENOCARCINOMA IN SCREEN-DETECTED VERSUS CLINICALLY DETECTED CASES

H.M.E. Van Agt¹, K. Rozemeijer¹, I.M.C. De Kok¹, S. Naber¹, S.M. Matthijsse¹, E.E.L. Jansen¹, L.I.H. Overbeek², A.G. Siebers², F.J. Van Kemenade³, M. Van Ballegooijen¹

¹Erasmus MC, University Medical Center, Department of Public Health, Rotterdam (Netherlands), ²PALGA, the Nationwide network and registry of histo- and cytopathology in the Netherlands, Houten (Netherlands), ³Erasmus MC, University Medical Center, Department of Pathology, Rotterdam (Netherlands)

Background / Objectives

In international studies, the incidence of cervical squamous cell carcinoma has been found to decrease, whereas no changes or even increases were observed in the incidence of cervical adenocarcinoma. It has been suggested that the current screening practices are not sufficient to detect adenocarcinoma precursor lesions. We investigated whether incidence rates of squamous cell carcinoma and adenocarcinoma differ between screen-detected and clinically detected cancers in the Netherlands.

Methods

All cervical cancer cases diagnosed between 1997 and 2007 were included using data from the Dutch nationwide registry of histo- and cytopathology (PALGA) and the Netherlands Cancer Registry. Analyses were restricted to cancers in women aged 29-64 years old, being the ages of the screening program in the Netherlands. Trends in the incidence rates of squamous cell carcinoma and adenocarcinoma were evaluated by calculating the estimated annual percentage change (EAPC). To compare the two sets of trend data, we used a test of parallelism (Joinpoint Trend Analysis).

Results

Overall, screen-detected cancers comprised of 75% squamous cell carcinomas, 17% adenocarcinomas and 8% of other histological types, which was similar to clinically detected cancers (75%, 20% and 6%, respectively; $p=0.771$). In the period 1997-2007, both screen-detected squamous cell and adenocarcinoma increased (EAPC

= 8.5, 95% CI: 1.4, 16.0; and EAPC = 14.2, 95% CI: 5.2, 23.8, respectively; p=0.014). Clinically detected cancers initially increased during 1997-2002 (EAPC = 26.6, 95% CI: 6.4, 50.0), yet showed a (non-significant) decreasing trend during 2002-2007 (EAPC = -2.4, 95% CI:-12.5, 8.9), a pattern similar to squamous cell and adenocarcinoma (p=0.648).

Conclusion

Overall, the distribution of cervical squamous cell and adenocarcinoma is similar in screen-detected and clinically detected cancers. The trends in the incidence rates of screen-detected and clinically detected cases were similar for both cancer types. The results suggest that the current screening practice in the Netherlands equally detects squamous cell and adenocarcinoma .

OC 02-05

Stage distribution of cervical cancer after diagnosis of atypical glandular cells in cervical screening

P. Sparen¹, J. Wang¹, K.M. Elfstrom², B. Andrae¹

¹Dept. of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm (Sweden), ²Dept. of Laboratory Medicine, Karolinska Institute, Stockholm (Sweden)

Background / Objectives

In a nationwide cohort study we followed more than 3 million Swedish women for cervical screening, diagnosis of pre-cursors and risk of cervical cancer (Wang et al, 2016). Our aim was to assess the risk of cervical cancer after cytological diagnosis of atypical glandular cells (AGC), compared to high-grade (HSIL) and low-grade cervical lesions (LSIL), as well as a normal Pap smear. Women with AGC had a risk of incident cervical cancer that was higher than for HSIL for up to 6.5 years, and particularly for adenocarcinoma, while the chance of finding prevalent cancer was lower than for HSIL but higher than for LSIL. Since we did not have complete stage information in this large cohort study, we decided to utilize the case series from an ongoing nationwide Case-Control Audit, with complete information on clinical stage at diagnosis. Our aim was to assess the distribution of clinical stage for cervical cancer cases occurring after AGC, by main histology, and stratified on prevalent and incident cases of cervical cancer.

Methods

In the updated Swedish national Case-Control Audit all 4254 cases of invasive cervical cancer from 2002 to 2011 were clinically and histopathologically verified, and age-matched to 30 population-based controls in a nested case-control design. Screening histories were retrieved from the Swedish National Cervical Screening Registry (www.nkcx.se/index_e.htm). In this preliminary analysis we utilized 351 cases with a first diagnosis of AGC in screening. Data were summarized into contingency tables by clinical FIGO stage, main histologic types (squamous cxa or adenocarcinoma), and prevalent (<6 months between diagnosis of AGC and cancer) or otherwise incident cancer. Chi square tests were applied.

Results

Preliminary results show that the stage distribution of cervical cancer for women with AGC was 29%, 58%, and 13% for stages IA, IB, II+, respectively. Cancers with prevalent cases were shifted towards earlier stages, compared to incident cases

($p=0.01$), but when stratifying on histological type there was no statistically significant difference in stage distribution between prevalent and incident cases ($p=0.27$ and $p=0.08$ for adenocarcinoma and squamous cxca, respectively). Although the majority of cases were diagnosed at stage IB for both histological types, the proportions of stages IA and II+ were higher for squamous cxca than for adenocarcinoma ($p<0.001$).

Conclusion

We found that more than half of cxca cases after AGC were diagnosed at stage IB, and almost 1/3 were diagnosed at stage IA. For prevalent cases the stage distribution was further shifted towards early stages, although most of this shift seemed to be explained by the histologic type of the cancer.

References

Wang J, Andrae B, Sundström K, Ström P, Ploner A, Elfström KM, Arnheim-Dahlström L, Dillner J, Sparén P. Risk of invasive cervical cancer after atypical glandular cells in cervical screening: nationwide cohort study. *BMJ*. 2016 Feb 11;352:i276. doi: 10.1136/bmj.i276

OC 02-06

IMPLEMENTATION OF A 'HUB AND SPOKES' MODEL OF DELIVERY OF CERVICAL SCREENING IN RURAL MALAWI

C. Christine¹, **S. Kafwafwa**², **B. Kabota**², **D. Morton**², **H. Walker**³, **G. Walker**³, **L. Grant**⁴, **R. Ter Haar**⁵, **H. Cubie**⁴

¹Usher Institute for Population Health Sciences and Informatics, University of Edinburgh (United kingdom), ²Nkhoma CCAP Hospital, Nkhoma, Malawi (Malawi), ³Dept of Obstetrics & Gynaecology, Royal Infirmary of Edinburgh (United kingdom), ⁴Global Health Academy, University of Edinburgh (United kingdom), ⁵Nkhoma CCAP hospital, Nkhoma, Malawi (Malawi)

Background / Objectives

Cervical cancer is a major cause of female cancer death in sub-Saharan Africa: Malawi has the highest global incidence. A 'screen and treat' approach using visual inspection with acetic acid (VIA) has government support but actual screening provision is limited due to lack of infrastructure, trained personnel, and the cost and availability of gas for cryotherapy. Recently, thermo-coagulation (also known as cold coagulation) has been acknowledged as a safe and acceptable procedure in this setting. We describe the setting up of a screening programme using VIA and thermo-coagulation for the treatment of low grade lesions coupled with appropriate, sustainable pathways of care for women with high grade lesions and cancers in Nkhoma CCAP Hospital and ten associated health centres in central Malawi.

Methods

Following approvals from the Ministry of Health and from regional and village chiefs, clinics were set up, staff trained and educational resources in the local language developed. At Nkhoma Hospital screening has been integrated within an existing Reproductive Health Unit. Attendance at the Malawian Ministry of Health VIA course is a prerequisite for all providers and is supplemented by additional theoretical and practical training in VIA interpretation and treatment using thermo-coagulation at Nkhoma Hospital. Bespoke standard operating procedures and assessment tools, and a training manual, were developed.

Results

13,424 previously unscreened women attended VIA clinics between October 2013 and September 2015. Screening clinics were held daily in the hospital and weekly in

eight health centres. In two health centres a mobile clinic was set up. Overall VIA positivity was 6.2%, but this varied by age, HIV status and clinic location. The majority of VIA-positive women received same-day treatment: in some health centres treatment is provided to VIA positive women on a monthly basis, and the need for partner consent also contributed to postponed treatment for a minority of women. A one-year cure rate of over 90% is observed, comparable to reported rates with cryotherapy. Women with suspected cancer at the health centres were referred to Nkhoma Hospital for further investigation.

Conclusion

Although high staff turnover and/or low staff levels provide challenges to maintenance of service levels in some health centres, a cervical screening programme has been set up, capitalising on already established trust between the hospital and health centres. Shared continued professional development sessions support an environment of mutual learning to strengthening the cadre of trained providers to implement the service more widely.

References

Campbell C et al. Use of thermo-coagulation as an alternative treatment modality in a 'screen and treat' programme of cervical screening in rural Malawi. IJC 2016; in press

OC 02-07

WHAT HAPPENS WHEN WOMEN IN A COUNTRY WITH ORGANISED CERVICAL CANCER SCREENING ARE NOT INVITED AS RECOMMENDED? AN OBSERVATIONAL STUDY

M.B. Larsen, B. Andersen

Randers Regional Hospital, dept. of Public Health Programmes (Denmark)

Background / Objectives

In 2013 it was uncovered that more than 19 000 women in the Central Denmark Region (CDR) had been unsubscribed from the Danish National Cervical Cancer Screening Programme (NCCSP) without a clear indication that this was intended, and therefore had not received invitations or reminders as recommended and announced by the authorities.

We report the essence of this unintended episode and aim to describe the outcomes of re-establishing invitations in terms of participation rate and screening results. Furthermore, we report on adjudications of compensations to women affected by the episode, and covering of the episode in the press.

Methods

The initiatives effectuated to rectify the error targeted three groups of women:

1. Women still in the screening age (23-64 years) were reassigned the programme
2. Women no longer in the screening age (above 64 years) were given the possibility of a final cervical cancer screening test (HPV testing)
3. Women diagnosed with Cancer Colli Uteri (CCU) after the date of the un-subscription were notified about the possibility to report the injury to the Danish Patient Compensation Association. If the women were no longer alive, their surviving relatives were contacted.

The proportion of women being tested within six months after invitation/last reminder was measured along with the corresponding screening result in terms of cytology or HPV test result. These data were obtained from the Danish Pathology Databank.

Information on number of patients reporting injuries due to unintended un-subscription, adjudications and the size of the compensations was continuously also obtained. Information of coverage of the episode in the press was obtained from a Danish media monitoring service.

Results

Of the 10 094 women still in the screening age, 37.7% had been tested despite no invitations. A total of 21.6% unsubscribed the programme again within one year, mainly because they did not want to participate (72.9%) or had total hysterectomy (24.8%). A total of 2 660 women were tested, 94.6% of the cytologies were normal, 1.5% showed HSIL and 0.04% showed cervical cancer.

Of the women above the screening age, 12.7% were tested. The proportion of women testing positive for HPV 16 or 18 was highest in the age groups 65-69 (1.4%) and 75-79 (1.9%).

Data on compensations and covering of the episode in the press will be presented at the conference.

Conclusion

This episode provides insight into the mechanisms of an organised screening programme showing that it has a life on its own independent of invitations. Furthermore, it shows that lacking invitations to screening are ranked alongside other adverse effects in the health care system.

OC 02-08

ROLE OF BIOMARKER TESTING FOR CERVICAL CANCER SCREENING IN A HIGH RISK POPULATION IN SOUTH AFRICA

W.W. Kremer¹, M. Van Zummeren¹, D.A.M. Heideman¹, R.D.M. Steenbergen¹, P.J.F. Snijders¹, G. Dreyer², C.J.L. Meijer¹

¹Department of Pathology, VU University Medical Center (Netherlands),

²Department of Obstetrics and Gynecology, University of Pretoria (South africa)

Background / Objectives

Cervical cancer remains one of South Africa's biggest women's health problems. Although there is a national screening policy, screening using cervical cytology is mainly done on an opportunistic basis reaching only a 13% coverage. Lack of accuracy and reproducibility of cytology screening combined with the low access to health care and the high incidence of HIV-positivity further reduces the effectiveness of cervical screening. Therefore new, objective diagnostic/screening methods should be evaluated. This study aims to evaluate the value of testing for several biomarkers, including hrHPV testing and DNA methylation marker testing, alone or in combination, as an alternative diagnostic/screening method to detect cervical cancer in a high risk South African population.

Methods

466 women have been enrolled in this cross-sectional study: 73 were referred for treatment because of HSIL, 37 were referred for clinical staging because of a newly diagnosed cervical carcinoma, and 356 were referred for cervical cancer screening because of their high risk due to HIV-positivity. Liquid-based cytology (LBC) samples were collected at the start of their visit (before treatment, staging or screening, respectively) for testing by cytology, hrHPV presence and DNA methylation of CADM1, MAL and miR124-2 genes. A colposcopy-directed biopsy was taken in all women who came for screening. Performance of cytology, hrHPV and methylation marker testing for detection of CIN3+ was compared.

Results

Presently, some data of 405 women have been collected. In the HIV+ screening population (n=356), primary hrHPV testing showed a high sensitivity for CIN3+ (90.3% (95% CI 79.9% - 100%)), at a specificity of 60.5% (95% CI 54.5% -

66.4%). Cytology revealed lower sensitivity (74.2% (95% CI 58.8% - 89.6%), but higher specificity (78.7% (95% CI 73.7% – 83.7%). Methylation analyses at different threshold settings are currently being performed. Preliminary data indicate that all women with cervical cancer score methylation positive. Further data and combined analyses of the various markers will be presented.

Conclusion

In this high risk population, sensitivity of primary hrHPV testing for the detection of CIN3+ is high, but at the cost of a low specificity. Cytology had lower sensitivity but higher specificity for CIN3+ testing. Preliminary results show that full molecular testing for CIN3+ in a low/middle income country is feasible.

OC 02-09

A MODEL APPROACH TO ASSESS BENEFIT OF HPV TESTING OVER CYTOLOGY IN SCREENING CERVICAL CANCER PRECURSOR

T. Tantitamit¹, W. Termrungruenglert², N. Khemapech², P. Havanond²

¹HRH Princess Maha Chakri Sirindhorn Medical Center (MSMC), Srinakharinwirot University (Thailand), ²King Chulalongkorn Memorial Hospital, Chulalongkorn University (Thailand)

Background / Objectives

Background: HPV DNA testing has emerged as an effective alternative option to current recommendation for cervical cancer screening with cytology. There is limited data on the efficacy of primary HPV DNA testing strategy in Thailand.

Objectives: The aim of this study is to compare the efficacy of HPV 16/18 genotyping test, high risk HPV DNA testing alone and liquid-based cytology method in screening cervical cancer precursor, using Markov model.

Methods

Population: The hypothetical cohort of 100,000 healthy women aged 30 to 65 years were simulated in each strategy.

Method: A Markov model was used to describe the course of total detected cases of CIN2⁺ over 35 years. Screening program started at age 30 and performed every five years' interval. The cohort model compared three strategies among HPV 16/18 genotyping test with reflex liquid based cytology triage, high-risk HPV testing alone followed by referral to colposcopy and cytology-based screening followed by referral to colposcopy. We assumed that the rate of lost follow-up of those referred to colposcopy would be 0%. The clinical parameter was estimated using the data from a recent prospective study of Thailand National Cancer Institute.

Results

Results: Of the three screening strategies that were evaluated, high risk HPV DNA testing alone was the most effective strategy for detection of CIN2⁺ over 35 years. It detected 143 and 510 cases per 100,000 women more than HPV 16/18 genotyping test and cytology-based strategy respectively. Compare the HPV 16/18 genotyping test and cytology-based; HPV 16/18 genotyping test detected 368 cases per 100,000 women more than cytology-based. In addition, every five years' interval, there were missed cases about half of detected cases screening by cytology strategy and 10% of detected cases screening by HPV 16/18 genotyping test.

Conclusion

Conclusion: This study strongly supports that HPV DNA testing is preferred to cytology-based screening for cervical cancer precursor. However, the balance between benefits, burden and cost of each screening program should be considered.

OC 02-10

Economic analysis of a strategy to improve cervical cancer screening in Norway: Cytology with pooled HPV triage vs. HPV genotyping with reflex CINtec PLUS Cytology triage

T. Ofstad¹, J.P.P. Kempers², S.W. Sørbye³

¹Roche Diagnostics Norge AS (Norway), ²Roche Diagnostics, PhD Health Economist (Netherlands), ³University Hospital of North Norway, PhD MD (Norway)

Background / Objectives

Healthcare decision makers are keen to implement cervical cancer (CC) screening strategies that produce better clinical outcomes, while controlling the cost. These choices are complex and evidence often incomplete. This modelling study facilitates this process by comparing clinical benefits and costs of replacing; 1) cytology with pooled HPV triage (current practice), with 2) cobas HPV genotyping and reflex CINtec PLUS Cytology triage (comparator) in the national CC screening program of Norway.

Sensitivity limitations of cytology and moderate specificity of pooled HPV testing, may require more frequent follow-ups or unnecessary invasive procedures. The cobas HPV test identifies women at increased risk for CC, by detecting the two highest-risk genotypes 16/18, which cause 70% of all CC. The CINtec PLUS confirms a transforming HPV infection by detecting cervical cells where HPV has disrupted cellular control (p16/Ki-67+). CINtec PLUS triage of HPV genotype 16/18+ predicts which women most likely have pre-cancerous cervical lesions and therefore benefit from an immediate colposcopy.

Methods

The Markov model compares clinical impacts and annual costs of the screening strategies. A hypothetical cohort of 836,000 Norwegian 25-65 year old women goes through two screening cycles in the model. Natural progression/regression of the disease is also modelled. In the current practice; cytology negative women return to routine screening, a reflex HPV test is done for ASCUS and LSIL results, and women with HSIL undergo a colposcopy. In the comparator strategy; HPV negative women return to routine screening, a reflex CINtec PLUS done for HPV genotypes other than 16/18, and women with the high risk genotypes 16/18 undergo a colposcopy. The screening intervals are 3 years in the current and 5 years in the comparator strategy. Test sensitivity and specificity data are from the ATHENA study¹. Other inputs include prevalence of HPV, HPV genotypes 16/18, abnormal cytology, CIN and CC in

Norway. Screening, diagnosis and CC treatment costs are calculated from a healthcare provider's perspective in 2015.

Results

The comparator strategy increases the detection of CC cases from 50.7% to 90.2% and reduces annual incidence of CC in the screened population from 154.8 to 71.9. Importantly, it reduces annual costs by 4%, from 145 to 139 million NOK, which is driven by the longer screening interval and averted CC treatment costs.

Conclusion

The results suggest that replacing cytology with pooled HPV triage, with cobas HPV primary screening with genotyping and reflex CINtec PLUS Cytology triage, in the national cervical cancer screening program of Norway produces better clinical outcomes and saves costs.

References

1. Castle, P., Stoler, M., Wright, T., Sharma, A., Wright, T., & Behrens, C. (2011). Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *The Lancet Oncology*, 12(9), 880-890. [http://dx.doi.org/10.1016/s1470-2045\(11\)70188-7](http://dx.doi.org/10.1016/s1470-2045(11)70188-7)

OC 03-01

LONG-TERM PROTECTION OF VIRUS-LIKE PARTICLE (VLP)-BASED HUMAN PAPILLOMAVIRUS (HPV) VACCINES

A. Saah¹, D. Brown², M. Pillsbury¹, A. Luxembourg¹, A.S. Kulkarni¹

¹Merck Sharp & Dohme, Corp. (United States of America), ²Indiana University School of Medicine (United States of America)

Background / Objectives

Long-term studies have demonstrated continued protection against vaccine-specific HPV types present in licensed VLP-based quadrivalent (6/11/16/18) and bivalent vaccine (16/18) for up to 9 years.^{1,2} However, a limited number of long-term studies and analyses of clinical studies have shown that cross-protection post-vaccination for non-vaccine types is partial at best, and markedly wanes over time.³⁻⁵ As such, the 9-valent vaccine may provide consistent and long-term protection against the 9 types in the vaccine. This analysis was undertaken to estimate the impact of VLP-based HPV vaccination on HPV infection as related to vaccine-specific types over time.

Methods

The effects of quadrivalent (types 6/11/16/18) and 9-valent (types 6/11/16/18/31/33/45/52/58) vaccines on HPV types 16/18/31/33/45/52/58-related incidences of cervical cancer, cervical intraepithelial neoplasia (CIN) 1 and CIN2/3 were compared. A model based on clinical trial data from the vaccines in >16,000 females of up to 6 year durations was used to estimate protection over time.

Results

The incidences of cervical cancer, CIN1 and CIN2/3 were reduced for the HPV-related vaccine types 16 and 18 for the quadrivalent vaccine, and for types 16/18/31/33/45/52/58 for the 9-valent vaccine. The 9-valent vaccine reduced cases of cervical cancer by an additional 21%, CIN1 by an additional 38%, and CIN2/3 by an additional 33%, compared with the quadrivalent vaccine, as estimated through 100 years.

Conclusion

Vaccination with the VLP-based HPV quadrivalent and 9-valent vaccines is expected to provide consistent and long-term protection against infection and disease caused by HPV types represented in the VLP-based vaccine. This includes a high level of type-specific protection against the HPV types represented in the 9-valent HPV vaccine (6/11/16/18/31/33/45/52/58).

References

1. Kjaer SK, Nygard M, Dillner J et al. Long-Term Effectiveness and Safety of Gardasil™ in the Nordic Countries. European Research Organization on Genital Infection and Neoplasia February 4-7. OC 6-1 (2015). Available at http://www.eurogin.com/2015/images/pdf/eurogin-2015_abstracts_part_2.pdf (accessed 21 Dec 2015).
2. Naud PS, Roteli-Martins CM, De Carvalho NS et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. *Hum. Vaccin. Immunother.* 2014; 10: 2147-62.
3. Malagon T, Drolet M, Boily MC, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect. Dis.* 2012; 12: 781-89.
4. Brown D, Kulkarni A, Matt Pillsbury et al. Clarification on the Impact of Cervarix Vaccination on Human Papillomavirus Infection and Cervical Cancer in the United Kingdom. *Hum. Vaccin. Immunother*, 2016; in press.
5. Kreimer AR, Struyf F, Rosario-Raymundo MR et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica Vaccine and PATRICIA trials. *Lancet Oncol.* 2015; 16: 775-86.

OC 03-02

**PERSISTENCE OF IMMUNE RESPONSE 10 YEARS
AFTER ADMINISTRATION OF THE HUMAN
PAPILLOMAVIRUS (HPV)-16/18 AS04-ADJUVANTED
VACCINE TO WOMEN AGED 15–55 YEARS**

T.F. Schwarz¹, A. Galaj², M. Spaczynski³, J. Wysocki⁴, A.M. Kaufmann⁵, P.V. Suryakiran⁶, N. Folschweiller⁷, F. Thomas⁷, L. Lin⁷, F. Struyf⁷

¹Stiftung Juliusspital, Central Laboratory and Vaccination Centre, Würzburg (Germany), ²NZOZ Vitamed, Bydgoszcz (Poland), ³Poznan University of Medical Sciences, Department of Gynecologic Oncology, Poznan (Poland), ⁴Poznan University of Medical Sciences, Department of Preventive Medicine, Poznan (Poland), ⁵Charité-Universitätsmedizin Berlin, Department of Gynecology, Berlin (Germany), ⁶GSK Pharmaceuticals India Ltd., Vaccine Value and Health Science, Bangalore (India), ⁷GSK Vaccines, Clinical Research and Development, Wavre (Belgium)

Background / Objectives

This extended follow-up study evaluated long-term immunogenicity and safety of HPV-16/18 AS04-adjuvanted vaccine up to 10 years (Y) after administration of first vaccine dose in women aged 15–55Y.

Methods

This phase III, multi-centre, parallel, open-label study (NCT00947115) included women aged 15–25Y, 26–45Y and 46–55Y at the time of vaccination who received 3 doses of HPV-16/18 vaccine (at month 0,1,6) in study NCT00196937. Serum anti-HPV-16/18 antibody responses were assessed by ELISA and compared with natural infection¹ and plateau levels². Anti-HPV-16/18 antibodies were also assessed (by ELISA) in cervico-vaginal secretion (CVS) samples collected annually from volunteers and correlations with serum antibodies were determined. Fatal or vaccine-related serious adverse events (SAEs) were recorded throughout the study.

Results

10Y after first vaccination, persistence of antibodies was observed in initially seronegative women of all age groups, with seropositivity rate $\geq 96.3\%$ (anti-HPV-16) and $\geq 83.8\%$ (anti-HPV-18). Geometric mean titres (GMTs) tended to decrease with increasing age at vaccination (anti-HPV-16 GMT=965.4 [95%CI: 802.2, 1161.8] in

15–25Y-olds [N=123], 334.4 [270.5, 413.5] in 26–45Y-olds [N=121], 157.4 [128.4, 193.1] EL.U/mL in 46–55Y-olds [N=107]; anti-HPV-18 GMT=321.1 [95%CI: 265.0, 389.1] in 15–25Y-olds [N=127], 115.4 [93.9, 142.0] in 26–45Y-olds [N=142], 69.7 [56.0, 86.8] EL.U/mL in 46–55Y-olds [N=130]). In all age groups, at Y10, for both antigens, GMTs were at least 3.1-fold higher than those after natural infection. In 15–25Y-olds, anti-HPV-16 GMT remained 2.3-fold higher than the plateau at equivalent time points in efficacy studies in women 15–25Y, while anti-HPV-18 GMT appeared similar to the plateau level. In older age groups, GMTs were similar or below the plateau. Women with detectable antibodies in their CVS (positivity rate: 53.8–70.7% [anti-HPV-16]; 34.6–45.0% [anti-HPV-18]) tended to have higher serum antibody titres than women with no CVS antibodies detected (serum anti-HPV-16 GMT=1414.3 vs 512.0 [15–25Y], 749.6 vs 204.5 [26–45Y], 608.3 vs 134.3 [46–55Y]; anti-HPV-18 GMT=667.7 vs 294.1 [15–25Y], 418.9 vs 84.4 [26–45Y], 334.9 vs 78.8 [46–55Y]). Correlations between antibody titres in serum and CVS were: R=0.6399 (anti-HPV-16), R=0.3819 (anti-HPV-18). 1 woman HPV-16-seropositive pre-vaccination reported cervical dysplasia and 2 reported fatal SAEs, not vaccine-related (leukaemia [1/194 at 45Y], lung neoplasm [1/171 at 62Y]).

Conclusion

Ten years after first vaccination, sustained immunogenicity of the HPV-16/18 vaccine was demonstrated in women aged 15–55 years at vaccination, with an acceptable safety profile.

Funding: GlaxoSmithKline Biologicals SA

References

(¹Paavonen et al. *Lancet* 2007; ²Naud et al. *Hum Vaccin Immunother* 2014)

OC 03-03

A LONG-TERM EFFECTIVENESS, IMMUNOGENICITY, AND SAFETY STUDY OF GARDASIL™ (HUMAN PAPILLOMAVIRUS [TYPES 6,11,16,18] RECOMBINANT VACCINE) IN YOUNG MEN (V501-020)

R. Das, A. Saah

Merck Sharp & Dohme, Corp. (United States of America)

Background / Objectives

Human papilloma virus (HPV) infection in men causes anogenital warts, anal cancer, and a proportion of penile cancers. Men are the main source of HPV infection in women. The V501-20 base study demonstrated the efficacy, immunogenicity, and safety of GARDASIL™ in young men. The extension of V501-020 evaluates the immunogenicity, safety and effectiveness of GARDASIL™ in preventing vaccine-type genital warts, external genital lesions (EGL), and anal intraepithelial neoplasia (AIN)/cancer in 16 to 26 year old men for 10 years after vaccination.

Methods

The V501-020 base study was a double-blind, placebo-controlled, multicenter, international study, in which young men were randomized 1:1 to receive GARDASIL™ or placebo. Subjects in the placebo group were offered catch-up vaccination. All subjects who received at least one dose of GARDASIL™ in the base study (early vaccination group, EVG) or thereafter (catch-up vaccination group, CVG) were followed annually in this extension. This interim analysis was performed 8 years post-vaccination.

Results

936 subjects in the EVG were followed for a median duration of 8.9 years after receipt of the first vaccine dose; 867 CVG subjects were followed for 4.2 years. No cases of HPV 6/11 genital warts or HPV 6/11/16/18 EGL were observed in the EVG per-protocol population during the extension. In a subpopulation evaluated for AIN, no high-grade disease and a single case of AIN1 was observed (0.3/100 person-years-at-risk, compared to 5.8 per 100 person-years-at-risk in the base study). Seropositivity rates for HPV 6/11/16/18 remained high and no vaccine-related serious adverse experiences were reported.

Conclusion

Vaccination with GARDASIL™ is immunogenic, well-tolerated, and provides durable protection from vaccine-type genital warts, EGLs, and AIN up to ~9 years following administration in 16 to 26 year-old men.

OC 03-04

TOLERABILITY OF A TWO-DOSE SCHEDULE OF THE BIVALENT HPV VACCINE

T.M. Schurink-Van 't Klooster¹, J.M. Kemmeren¹, R. Donken², N.A. Van Der Maas², H.E. De Melker²

¹Department National Immunisation Programme, Centre of Epidemiology and Surveillance of Infectious Diseases, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands (Netherlands), ²1Department National Immunisation Programme, Centre of Epidemiology and Surveillance of Infectious Diseases, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands 2Department of Pathology, VU University Medical Centre, Amsterdam (Netherlands)

Background / Objectives

After the change from a three-dose (0, 1 and 6 months) to a two-dose schedule (0 and 6 months) for HPV vaccination in the Netherlands in 2014, tolerability among girls was monitored and compared with the tolerability after the three-dose schedule measured in 2010.

Methods

Local and systemic adverse events (AEs) occurring within 7 days following each dose were obtained by online questionnaires. We also obtained online questionnaires on symptoms occurring in the week before vaccination.

Results

Following the two-dose schedule, girls reported local reactions in 86.1% (1st dose) and 82.0% (2nd dose) compared to 88.5% (1st dose), 79.5% (2nd dose) and 79.2% (3rd dose) in girls receiving three doses. For systemic AEs reported frequencies were 73.0% (1st dose) and 71.6% (2nd dose) compared with 85.9% (1st dose), 74.5% (2nd dose) and 75.8% (3rd dose). Overall, the two-dose schedule resulted in 32.0% less local reactions and 38.8% less systemic AEs than the three-dose schedule. The most reported local reactions were pain and reduced use of the arm. With respect to the systemic AEs, myalgia, fatigue and headache were the most reported. However, symptoms such as fatigue, headache and cold, were reported similar or even more in the week before compared to the week after vaccination in girls who had received the

two-dose schedule. This data was in line with symptoms reported by girls before the third dose of the three-dose schedule.

Conclusion

Local and systemic AEs were frequently reported within 7 days following HPV vaccination in a two-dose schedule. The tolerability of the first dose was more favourable compared with the first dose of the former three-dose schedule. For the dose at six months, the tolerability was comparable for both schedules. In conclusion, the change to a two-dose schedule resulted in considerable fewer AEs, which potentially improves acceptance of vaccination.

OC 03-05

No evidence of type replacement following HPV16/18 vaccination: Pooled analysis of data from the Costa Rica Vaccine and PATRICIA randomized trials

J. Tota¹, D. Bi², F. Struyf², M. Merikukka³, A. Hildesheim*¹, M. Lehtinen*⁴

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute (United States of America), ²GSK Vaccines (Belgium), ³National Institute for Health and Welfare (Finland), ⁴University of Tampere, School of Public Health (Finland)

Background / Objectives

Current HPV vaccines do not protect against all oncogenic HPV types. Following vaccination type replacement may occur, especially if different HPV types competitively interact during natural infection. There is little evidence that HPV type competition exists, but it is difficult to assess type interactions in observational studies. Randomized controlled trials (RCTs), originally designed to evaluate HPV vaccine efficacy, provide a good setting to evaluate type replacement.

Methods

Data were pooled from the Costa Rica Vaccine Trial (CVT; NCT00128661) and the PATRICIA trial (NCT001226810) – two large-scale, double-blind RCTs of the HPV-16/18 AS04-adjuvanted vaccine – to compare cumulative incidence of non-protected HPV infections after 4 years of follow-up (oncogenic types 35, 39, 51, 52, 56, 58, 59, 68/73; non-oncogenic types 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, 74). In both trials, HPV DNA testing was conducted using the SPF10/DEIA - LiPA25 version 1 method and type-specific PCR for HPV16/18. In our primary analyses, incident infection was defined as an infection during follow-up (single time detection) that was not present during the vaccination phase (entry/6-month visits), or if present, that cleared during follow-up (single time clearance) before reappearing. Females were not considered at risk while infected with HPV types that the HPV-16/18 vaccine is suspected to confer protection against (HPVs 6, 11, 16, 18, 31, 33, 45, 51, and 74). These types were excluded from our grouped analyses of oncogenic and oncogenic/non-oncogenic HPV types, but we considered HPVs 6, 11, 51, and 74 in our individual-type analyses. Negative rate difference estimates were interpreted as evidence of type replacement if the associated 95% CI excluded zero.

Results

After applying relevant exclusion criteria, 21,596 females (age 15 to 25 years) were included in our pooled analysis (HPV arm = 10,750, control arm = 10,846). Incidence rates (per 1000 infection-years) were lower in the HPV arm than in the control arm for grouped oncogenic HPV types (rate difference = 1.6, 95% CI = 0.9 to 2.3) and for grouped oncogenic/non-oncogenic types (rate difference = 0.2, 95% CI = -0.3 to 0.7). Focusing on individual HPV types separately, no deleterious effect was observed for any of the 20 types we evaluated; however, a nominal protective effect was observed against oncogenic HPV types 35, 52, 58, and 68/73, as well as non-oncogenic types 6 and 70.

Conclusion

HPV type replacement does not occur among vaccinated individuals within 4 years, and is unlikely to occur in populations comprised of vaccinated individuals.

Submitted on behalf of the CVT and PATRICIA study groups; *Joint senior authors

OC 03-06

Health and Economic Impact of Vaccinating Boys in Addition to Girls against Oncogenic HPV in the Netherlands

V. Qendri¹, J.A. Bogaards², J. Berkhof¹

¹Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam (Netherlands), ²Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven (Netherlands)

Background / Objectives

Most of the developed countries, including the Netherlands, have adopted a national HPV immunization program for girls. However, the number of preadolescent girls in the Netherlands who have completed the full vaccine series has plateaued around 58-60%. We examined the relative health impact of vaccinating boys in comparison to increasing the HPV vaccination coverage among girls, and performed a cost-effectiveness analysis of a sex-neutral vaccination plan.

Methods

We expanded a Bayesian synthesis framework, previously employed to estimate the benefits in the burden of the HPV 16 and 18-related disease in men following a gender-neutral vaccination campaign (Bogaards et al BMJ 2015). Our model considered the full spectrum of the HPV related cancers in males and females and accounted for the herd immunity effect deriving from female and male vaccination. We explicitly distinguished the benefit in the male homosexual population from that in the heterosexual population. We assumed a 98% vaccine efficacy and lifelong protection against HPV 16 and 18-related disease. In the cost-effectiveness analysis we investigated whether vaccinating 40% of the boys is a good-value-for-money strategy in addition to the current vaccination coverage of 60% among girls. Costs and effects were discounted by 3%, the cost-effectiveness threshold was set equal to the gross domestic product per capita, and a health-care-decision-maker perspective was taken.

Results

We observed that vaccinating 40% of the boys yields the same gain in life-years as increasing the uptake in girls from 60 to 80%. Vaccinating 40% of boys in addition to 60% of girls gained an additional 5.2 life-years per thousand girls and 5.8 life-years per thousand boys. Prevention of cervical disease accounted for 73% of the life-year gain in girls, and 67% of the gain in boys was due to prevention of oropharyngeal cancers. Adding 40% uptake among boys was cost-effective, even at high total

vaccination cost of 200-300 euros per vaccinated boy. If we would have excluded oropharyngeal cancer from our calculations, vaccinating boys would be cost-effective at vaccination costs of 100-150 euros, which seems achievable at current low HPV vaccine tender prices.

Conclusion

Vaccinating boys is likely to be a cost-effective alternative if the uptake among girls remains at the current level of 60%.

References

Bogaards, J. A., Wallinga, J., Brakenhoff, R. H., Meijer, C. J., & Berkhof, J. (2015). Direct benefit of vaccinating boys along with girls against oncogenic human papillomavirus: bayesian evidence synthesis. *bmj*, 350, h2016.

OC 03-07

OVERALL IMPACT OF HPV VACCINATION STRATEGIES - A RANDOMIZED TRIAL

M. Lehtinen, On Behalf Of The Hpv-040 Study Group

University of Tampere, Finland, Karolinska Institute, Sweden (Finland)

Background / Objectives

Human papillomavirus (HPV) causes up to 5% of male and 10% female cancers. Prophylactic HPV vaccines are efficacious against precancerous cervical lesions but the impact of different vaccination strategies is unknown. Results of a community-randomized, controlled, partially blind phase (III)/IV study (HPV-040: NCT 00534638) on the co-primary end-point: overall effectiveness (comprising direct vaccine efficacy (VE) and indirect effectiveness) of gender-neutral and girls-only vaccination, and direct VE of HPV-16/18 AS04-adjuvanted vaccine are reported.

Methods

In 2007-10, 80.272 adolescents born 1992-5 from 33 randomized urban/semi-urban communities in Finland, a priori stratified into 12 low, 9 intermediate and 12 high HPV-16/18 seroprevalence were invited (1). In 11 Arm A communities 90% of vaccinated girls and boys received HPV-16/18 AS04-adjuvanted vaccine, in 11 Arm B communities 90% of vaccinated girls received the HPV-16/18 vaccine and all vaccinated boys received hepatitis B-virus (HBV) vaccine. In 11 Arm C communities all vaccinees received HBV-vaccine. HPV DNA prevalence in altogether 10.826 cervical and 4.871 oropharyngeal samples of HPV-16/18 vaccinated and non-HPV-16/18 vaccinated 18.5 year-old girls were determined by SPF10 and/or MALTI-DOF PCRs.

Results

Enrolment of 32.175 adolescents during immunization phase: 20.513 girls (51-53% participation rate) and 11.662 boys (22-32% participation rate) was uniform by birth cohort (1). The mobility among different communities between 15 and 18.5 years was low and comparable between arms. Smoking/alcohol consumption and sexual health characteristics were generally comparable in the different arms. Baseline HPV prevalence between arms was disproportionate, and there were no statistically significant differences between the overall effectiveness estimates of the HPV-16/18 vaccination strategies. The overall effectiveness of HPV-16/18 vaccination in all 18.5 year-old females for gender-neutral and girls-only vaccination strategies was respectively 24% (95%CI -19-51) and 50% (95%CI 20-68) based on stratified,

cluster-adjusted Mantel-Haenszel test, and 36% (95%CI 6-56) and 51% (95%CI 33-64) based on multivariate logistic regression adjusted for covariates. Direct VE against HPV-16/18 infection as measured by prevalence in the HPV-16/18 vaccinated 18.5 year-old females from the combined A and B vs. C arms was high against both HPV-16/18 genital infection (adj. VE 93.3%, 95%CI 87.7-96.4), and against oropharyngeal infection (adj. VE 82%, 95%CI 47-94).

Conclusion

Randomized trial-based data on the impact of different HPV vaccination strategies on HPV prevalence will hopefully soon guide implementation of vaccination programs.

References

Funding GlaxoSmithKline Biological SA

1. Lehtinen M, et al. Vaccine 33:1284-1290, 2015

OC 03-08

DECLINE IN QUADRIVALENT HUMAN PAPILLOMAVIRUS INFECTION IN YOUNG SEXUALLY ACTIVE HETEROSEXUAL MEN WITH CHLAMYDIA TRACHOMATIS 8 YEARS FOLLOWING THE UNIVERSAL AUSTRALIAN FEMALE VACCINATION PROGRAMME: IMPLICATIONS FOR HERD PROTECTION

E.P.F. Chow¹, **D. Machalek**², **S.N. Tabrizi**³, **J.A. Danielewski**², **G. Fehler**¹, **M.Y. Chen**¹, **C.S. Bradshaw**¹, **S.M. Garland**³, **C.K. Fairley**¹

¹Melbourne Sexual Health Centre, Alfred Health, Melbourne (Australia),
²Murdoch Childrens Research Institute, Parkville (Australia), ³Department of Microbiology and Infectious Diseases, The Royal Women's Hospital, Parkville (Australia)

Background / Objectives

Australia introduced the national quadrivalent human papillomavirus (4vHPV) vaccination programme in mid-2007 in young women. The aim of this study was to examine the trend in prevalence of vaccine targeted HPV types contained in 4vHPV and the nine-valent (9vHPV) vaccines among sexually active heterosexual men, and to investigate whether men are protected by herd protection from the universal female vaccination programme.

Methods

A total of 1,466 heterosexual men aged ≤ 25 attending Melbourne Sexual Health Centre between 1 July 2004 and 30 June 2015 diagnosed with *Chlamydia trachomatis* were included. Detection of HPV genotypes was performed using the PapType high-risk HPV detection and genotyping kit on stored urine or urethral swab samples. The prevalence of any HPV types, 4vHPV types (6/11/16/18) and the additional types present in the 9vHPV (31/33/45/52/58 alone) were calculated for each Australian financial year and stratified by age, vaccine eligibility, and country of birth.

Results

The proportion of samples with 4vHPV types dramatically dropped from 20% [95% CI: 10-33%] in 2004/05 to 3% [0-11%] in 2014/15 ($p_{\text{trend}} < 0.001$) among 633 Australian-born men; and a greater decline was observed in 237 Australian-born men aged ≤ 21 years old (from 31% [9-61%] to 0% [0-14%]; $p_{\text{trend}} < 0.001$) in the last 11 years. No temporal changes were observed in any HPV types and in types 31/33/45/52/58 in Australia-born men.

A significant decline in HPV 16/18 (from 18% [6-37%] to 4% [1-9%]; $p_{\text{trend}} = 0.014$), but not in HPV 6/11 (from 11% [2-28%] to 4% [1-9%]; $p_{\text{trend}} = 0.319$) was seen among overseas-born men. Of the 258 overseas-born men who arrived in Australia from a country that introduced the bivalent vaccine programme (predominantly the UK), the prevalence of HPV16/18 dropped from 17% [6-36%] to 10% [6-14%] between pre- and post-vaccination period ($p = 0.017$), but there was no change in HPV 6/11 (from 7% [1-23%] to 6% [3-10%]; $p = 0.260$). There was also no change in 4vHPV types in men from a country without a vaccination programme.

Conclusion

There has been a fall in prevalence of 4vHPV among unvaccinated Australia-born men, suggests men receive herd protection from the universal female programme. In addition, the decline in HPV 16/18, but not HPV 6/11 in overseas-born males predominantly from a country with a bivalent vaccine programme, suggests these men receive benefits from herd protection for 16/18 from their vaccinated female partners in their own countries.

OC 04-01

IMMEDIATE REFERRAL TO COLPOSCOPY VS. CYTOLOGICAL SURVEILLANCE FOR LOW-GRADE CERVICAL CYTOLOGICAL ABNORMALITIES IN THE ABSENCE OF HPV-TEST: A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE LITERATURE

M. Kyrgiou¹, I. Kalliala¹, M. Arbyn², B. Ng³, P. Martin-Hirsch⁴, E. Paraskeva⁵

¹Institute of Reproduction and Developmental Biology, Department of Surgery & Cancer, Imperial College London (United kingdom), ²Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels. (Belgium), ³Department of Obstetrics and Gynaecology, University Hospital Southampton NHS Foundation Trust (United kingdom), ⁴Department of Gynaecologic Oncology, Lancashire Teaching Hospitals, Preston. (United kingdom), ⁵Department of Obstetrics and Gynaecology, University of Ioannina, 45500, Ioannina (Greece)

Background / Objectives

Minor cytological abnormalities in primary cervical screening are common and often difficult to manage. The aim of this review was to explore the optimum management strategy for women with ASCUS or LSIL cytological abnormalities at primary screening in the absence of HPV DNA test.

Methods

We conducted a comprehensive literature search for randomised controlled trials comparing immediate colposcopy to cytological surveillance in women with borderline nuclear changes or low-grade dyskaryosis. The main outcomes studied were the default rates and the histological status of biopsies within immediate colposcopy compared to biopsies taken on completion of surveillance. We calculated pooled relative risks (RR) and 95% confidence intervals using random-effects model and assessed inter-study heterogeneity using Cochrane's Q-test and I²-test.

Results

Six RCTs met the inclusion criteria. Compliance to follow-up declined over time and was significantly lower than to immediate colposcopy throughout the follow-up, RR 3.85, 95% CI 1.27-11.63, already at 6 months. Incidence of any CIN as well as

incidence of CIN 3 was significantly higher after immediate colposcopy compared to surveillance after 12 months, RR 1.72, 95% CI 1.09-2.70 and RR 1.63, 95% CI 1.25-2.12, respectively. After 24 months of surveillance only the incidence of low-grade lesions was significantly elevated after immediate colposcopy compared to surveillance: RR for any CIN 2.02, 95% CI 1.33-3.08, and for CIN 1, RR 2.58, 95% CI 1.69-3.94. Incidence of clinically more significant high-grade lesions was not statistically significantly elevated after same surveillance period: RR for CIN 2+ 1.14, 95% CI 0.66-1.97, and for CIN 3+ 1.02, 95% CI 0.53-1.97.

Conclusion

The higher incidence of low-grade CIN detected by immediate colposcopy might be explained by spontaneous regression of these lesions and could lead to unnecessary interventions and overtreatment. We did not observe significant differences in CIN 2+ or CIN 3+ incidence between immediate colposcopy and cytological surveillance. However, the risk of default from cytological surveillance significantly increased over time, which may increase the defaulters' risk of invasive cervical cancer.

OC 04-02

THE “IMPROVE-COLPO” STUDY ON USA COMMUNITY-BASED COLPOSCOPY WITH DYNAMIC SPECTRAL IMAGING: DESIGN AND FIRST FINDINGS

E. Papagiannakis¹, A. Cholkeri-Singh², C.G. Olson³

¹DySIS Medical Ltd, Livingston (United kingdom), ²Dept of Ob/Gyn, Advocate Lutheran General Hospital, Park Ridge, IL; Dept of Ob/Gyn, University of Illinois at Chicago, Chicago, IL; The Advanced Gynecologic Surgery Institute, Naperville, IL (United States of America), ³Women's Center for Health, Naperville, IL and Loyola University Health Systems Maywood, IL (United States of America)

Background / Objectives

Colposcopy and directed biopsy often miss high-grade cervical intraepithelial neoplasia (CIN2+), impacting patient care and healthcare expenditure. We describe the ongoing IMPROVE-COLPO study that aims to chart routine US community-based colposcopy. The study quantifies and compares differences in practice, clinical and financial impact after adoption of a commercial digital colposcope that offers the adjunctive Dynamic Spectral Imaging (DSI), which quantifies and maps cervical acetowhitening. The integration of DSI in colposcopy was shown to improve CIN2+ detection in studies [1] and routine practice [2] in Europe.

Methods

IMPROVE-COLPO is a sponsored, multi-center, observational, IRB-approved two-arm community colposcopy study. The study recruits women ≥ 21 years old referred for colposcopy following current guidelines and practice at 42 facilities across the USA. A prospective arm collects outcomes with DSI used at the colposcopists' discretion for assessment and biopsy placement. A second arm collects colposcopist-matched retrospective outcomes of standard colposcopy. The primary measure is detection of CIN2+.

Results

The study has recruited more than 3300 subjects to date, of which the data for 1406 prospective and 1352 retrospective subjects are available for analysis. The baseline characteristics (age, race/ethnicity, menopausal status, insurance status and referral reason) of the study population are comparable between the two arms. We present the first findings on subjects referred with low-grade (LG) abnormalities, 1147 in the

prospective and 1145 in the retrospective arm. Of these patients, 84.8% underwent the procedure with histological outcome and confirmed the presence of CIN2+ in 9.2% of the population, including 8 invasive carcinomas. Colposcopic impression was found inconsistently recorded in the retrospective arm, and a poor predictor in the prospective arm, where only 6.8% of the subjects with CIN2+ were predicted correctly by colposcopists. The prediction of CIN2+ reached 75.4% after incorporating the DSI reading. The yield of detecting CIN2+ subjects by directed biopsy (True Positive Rate) increased by 32.7% in the prospective versus the retrospective arm, with a minimal increase of the False Positive Rate by 1.0%. The Positive Predictive Value (PPV) of directed biopsy also increased by 25.6% (35.2% for DSI-assisted biopsies).

Conclusion

The subject characteristics across the two study arms are comparable. Among LG referrals, the adjunctive use of DSI in the prospective arm has increased the CIN2+ detection rate and the PPV of directed biopsies when compared to the retrospective arm.

References

1. Louwers, J.A., et al., Dynamic spectral imaging colposcopy: higher sensitivity for detection of premalignant cervical lesions. *BJOG: An International Journal of Obstetrics & Gynaecology*, 2011. 118(3): p. 309-318.
2. Coronado, P.J. and M. Fasero, Colposcopy combined with dynamic spectral imaging. A prospective clinical study. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 2016. 196: p. 11-16.

OC 04-03

ANGLE-RESOLVED LOW COHERENCE INTERFEROMETRY (A/LCI) AS A NOVEL OPTICAL IMAGING TECHNOLOGY TO DETECT CERVICAL DYSPLASIA

D. Ho ¹, T. Drake ², A.B. Moscicki ³, K. Smith-Mccune ⁴, T. Darragh ⁴, A. Wax ¹, L. Hwang ⁴

¹Duke University (United States of America), ²Austin Community College (United States of America), ³University of California Los Angeles (United States of America), ⁴University of California San Francisco (United States of America)

Background / Objectives

Cytology, colposcopy, and cervical biopsy are the traditional mainstays for detection of cervical dysplasia but have some limitations in performance. Our long-term objective is to develop a novel optical imaging platform for the detection of cervical dysplasia. As a first step, we have adapted techniques in angle-resolved low coherence interferometry (a/LCI) for clinical application to the cervix. Our current study aim is to evaluate the ability of an a/LCI prototype device to distinguish CIN-1 and CIN-2/3 from healthy cervical tissue.

Methods

The a/LCI prototype is a hand-held polycarbonate wand with a point-probe tip placed against the cervix to deliver polarized light, producing a scan at subcellular resolution. The current in vivo study enrolled 40 women. For 33 patients referred to the UCSF Dysplasia Clinic for abnormal cytology, we performed routine colposcopy-directed biopsies and obtained a/LCI scans at each cervical site immediately prior to biopsy. For 7 healthy women recruited from another HPV cohort study, we obtained random cervical biopsies with a/LCI scans at each biopsy site. The a/LCI scans were examined to find the scattering profiles obtained at 200-250um below the tissue surface, in order to estimate the nuclear diameter and nuclear density at the basal layer of the biopsy site. These nuclear measurements obtained by a/LCI were compared between the healthy, CIN-1, and CIN-2/3 biopsies (as diagnosed by histology), using ANOVA and post hoc statistical methods.

Results

Scans from 31 women were of sufficient quality for analyses, yielding 63 cervical biopsy sites (Table 1). For both nuclear diameter and nuclear density, significant differences were found between CIN-2/3 versus healthy, and CIN-1 versus healthy tissue (all p values <0.01). CIN-2/3 also showed trends for increased nuclear diameter and nuclear density compared to CIN-1 (p=0.053 and 0.062 respectively)

Table 1. Nuclear measurements from a/LCI scans performed at 63 cervical biopsy sites			Conclusion
Histology Result	Nuclear Diameter by a/LCI, mean (standard deviation)	Nuclear Density by a/LCI, mean (standard deviation)	
Healthy (n=33)	8.22 um (0.83)	1.053 (0.005)	This initial study suggests that a/LCI technology holds promise as a modality for detection
CIN-1 (n=17)	11.30 um (0.77)	1.045 (0.005)	
CIN-2/3 (n=13)	12.04 um (0.96)	1.041 (0.003)	

of cervical dysplasia. Our on-going studies involve further iterations of this device. Our long-term goal is to develop additional applications of LCI technology, including multiplexed LCI (mLCI) to map the cell types of the cervical epithelium, and multi-modal LCI which combines these modalities into a single integrated platform for real-time clinical screening and diagnosis of cervical dysplasia.

OC 04-04

REGRESSION AND PROGRESSION PREDICTORS OF CIN2 IN WOMEN

D. Loopik¹, **S. Doucette**², **R. Bekkers**¹, **J. Bentley**³

¹Department of Obstetrics and Gynecology, Radboudumc, Nijmegen (Netherlands), ²Research Methods Unit, Nova Scotia Health Authority, Halifax, Nova Scotia (Canada), ³Department of Obstetrics and Gynecology, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia (Canada)

Background / Objectives

Background: The Society of Obstetricians and Gynaecologists of Canada and the Society of Canadian Colposcopists (SOGC/SCC) established a new guideline in 2012 with the advice to manage women under the age of 25 years with cervical intraepithelial neoplasia grade 2 (CIN2) with repeat colposcopy and cytology at 6-months intervals for up to 24 months before treatment is considered [1]. This advice was based on some small studies showing regression in approximately 60% of cases.

Objective: The purpose of this study was to review the management and outcome of histologically confirmed CIN2 in women under the age of 25 years.

Methods

A retrospective review was performed, investigating women less than 25 years old at the time of diagnosis with biopsy proven CIN2 between January 1, 2010 and December 31, 2014 who were seen in the colposcopy clinic at the Queen Elizabeth II Hospital in Halifax, Nova Scotia, Canada.

The regression, persistence and progression rate of CIN2 in conservatively managed women were evaluated and potential risk factors were examined. Colposcopic, cytologic and histologic findings of women with conservative management were compared to women who underwent immediate treatment (<6 months).

Results

Of the 319 women with biopsy proven CIN2, 108 women received immediate treatment and 211 women were managed conservatively. Of these 211 women, 144 remained untreated while 67 women received treatment ≥ 6 months after initial colposcopy.

In total 150 (71.1%), of the 211 women managed conservatively, showed regression, while 26 women (12.3%) had persistent disease, and 35 women (16.6%) progressed. Their median follow-up time was 15.1 months. None of the women included in the study progressed to invasive cancer. Smoking was identified as a risk factor for

progression with a hazard ratio of 2.4 (p=0.006).

In the immediate treatment group 30 (27.8%) of the 108 women being treated had CIN1 or less as histologic result, compared to 15 (7.1%) of the 211 women from the observation group.

Conclusion

Based on the 71.1% regression rate within a median follow-up of 15.1 months, a conservative approach of CIN2 is the preferred management option for women under the age of 25 years. Unnecessary treatment may be avoided by a conservative approach, preventing adverse effects on obstetric outcome. Smoking was identified as a risk factor for progression.

References

1. Bentley J; Society of Canadian Colposcopists. Colposcopic Management of Abnormal Cervical Cytology and Histology. J Obstet Gynaecol Can 2012;34:1188–1202.

OC 04-05

VAGINAL ECOSYSTEM CHANGES AND PERSISTENCE/RECURRENCE OF THE CERVICAL PRECANCEROUS LESIONS IN PATIENTS IN LATVIA.

I. Jermakova, D. Rezeberga, J. Žodžika, O. Plisko

1- Riga Stradiņš University, 2-Riga East Clinical University Hospital (Latvia)

Background / Objectives

Vaginal pH is related to changes in the bacterial flora, both suggested cofactors for persistence of cervical intraepithelial neoplasia (CIN) . (1,2)

Methods

We performed pH measurement and native microscopy of vaginal samples obtained from 50 women aged 18-65 with abnormal cytology referred for colposcopy during their first visit to Reference Colposcopy Centre in Riga East Clinical University Hospital in 2013. Vaginal pH was measured using Machery Nagel pH strips. Microscopic examination of wet mounts was interpreted according to Donders' modification of Schröders' classification. We then analysed these patients case reports retrospectively in 2014-2015. In patients with high grade CIN diagnosed during 1st visit, cytology and colposcopy are performed during their 2nd/3rd visit after excisional treatment and in patients with primary diagnosed low grade CIN, colposcopy and punch biopsy are carried out during follow up.

Results

Elevated pH >4.4 was detected in 8 patients with LSIL and 22 HSIL cytology cases. Lactobacillary grade I (LBG I) was associated with squamous intraepithelial lesions (SIL) changes only in 10 cases (LSIL-2 and HSIL-8), but the prevalence of LBG III was diagnosed in 20 cases (LSIL-4, HSIL-16) . Elevated pH>4.4 was reported in 23 of 37 patients with CIN2/3 in histology reports after excisional treatment and in 8 of these patients persistence or recurrence of the CIN was diagnosed during follow up. But normal pH ($pH \leq 4,4$) was more likely correlate with normal cytology/hystology

($p=0.01$) during 2-3rd visit: in these group of patients CIN persistence/recurrence was found only once.

Conclusion

A significant correlation between the increased vaginal pH level/abnormal vaginal LBG and persistence/recurrence of cervical precancerous lesions was found. Our findings confirm the association between pathological vaginal flora and persistence of cervical precancerous lesions. We are planning to study the role of correlation between vaginal flora patterns, cytokines activity and HRHPV oncoproteins activity in the process of CIN persistence and cancerogenesis.

References

1. Cervical intraepithelial neoplasia is associated with genital tract mucosal inflammation. *Sex Transm Dis.* 2012 Aug;39(8):591-7
2. Role of the vaginal microbiological ecosystem and cytokine profile in the promotion of cervical dysplasia: a case-control study. *Infect Dis Obstet Gynecol.* 2002;10(4):181-6.

OC 04-06

SPERANZA STUDY: PRELIMINARY RESULTS OF HPV VACCINATION AFTER LOOP ELECTROSURGICAL EXCISION PROCEDURE FOR CERVICAL INTRAEPITHELIAL NEOPLASIA

A. Ghelardi, P. Bay, A. Tonetti, A.F. Ragusa

Azienda USL Toscana nord ovest (Italy)

Background / Objectives

In 2006 HPV vaccination was licensed for primary prevention programs worldwide (1). Only a few years after, several studies have raised new scenarios about HPV related diseases with strong implications on clinical management in adult women (2). Some findings from retrospective data have shown a significant effect of HPV vaccine on women and men treated for HPV pathologies (3). Although vaccination is not effective in patients with ongoing HPV infection, some studies have suggested that HPV vaccine could influence the course of the disease after a surgical treatment (4). Reduction in disease relapse after treatment in vaccinated patients comes from gastroenterological, gynecological and dermatological evidences concerning both benign lesions (warts) and precancerous lesions (CIN, VIN, AIN) (5).

The aim of the study is to determine if HPV-vaccination post conization can decrease the rate of cervical disease recurrence in women treated with LEEP for CIN.

Methods

All women aged less than 46 years treated for CIN2+ in our gynecology unit were enrolled in a case-control prospective study. Case group received HPV quadrivalent vaccine post LEEP while control group was submitted to follow-up alone. Follow-up was performed every 6-months by colposcopy, and cytology in the first year after LEEP, after annually. Abnormal findings were histologically confirmed and considered as recurrence if CIN2+. Statistical analysis was performed by Pearson's chi squared test.

Results

In January 2013 Azienda USL-1 Massa Carrara has approved a clinic for HPV-vaccination. The first project of the clinic has been the SperAnZA study (Sperimentazione anti-HPV zona apuana). From a total of 398 enrolled patients we

present data of women undergoing at least 6-months follow-up period. The median follow-up time was 27 months. Women were equally assigned to the 2 groups; 11 out of 162 patients in control group developed a cervical recurrence (6%) while 2 out of 162 vaccinated women recurred (1%). The rate of recurrence was significantly higher in the control group, with a $p=0.0199$ by Pearson's chi squared test.

Conclusion

Our preliminary results indicate that quadrivalent HPV-vaccination after LEEP treatment for CIN may be useful in preventing recurrence of the disease. HPV vaccination could prevent subsequent new infection and prevent infection of the same variant virotype. This study is, at our knowledge, the only prospective clinical trial which analyses the effect of HPV vaccination after surgical treatment. The clinical implications of this new opportunity could be very strong, in order to influence post treatment management of HPV diseases.

References

- 1) Human papilloma virus vaccines: WHO position paper . Weekly epidemiological record. <http://www.who.int>
- 2) Evaluation of quadrivalent HPV 6/11/16/18 vaccine efficacy against cervical and anogenital disease in subjects with serological evidence of prior vaccine type HPV infection. Olsson SE, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, Perez G, Brown DR, Koutsky LA, Tay EH, Garcia P, Ault KA, Garland SM, Leodolter S, Tang GW, Ferris DG, Paavonen J, Lehtinen M, Steben M, Bosch FX, Dillner J, Joura EA, Majewski S, Muñoz N, Myers ER, Villa LL, Taddeo FJ, Roberts C, Tadesse A, Bryan J, Maansson R, Vuocolo S, Hesley TM, Saah A, Barr E, Haupt RM. *Hum Vaccin*. 2009 Oct;5(10):696-704.
- 3) Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease : retrospective pooled analysis of trial data. Joura EA, Garland SM, Paavonen J, Ferris DG, Perez G, Ault KA, Huh WK, Singhs HL, James MK, Haupt RM; FUTURE I and II Study Group. *BMJ*. 2012 Mar 27;344:e1401. doi: 10.1136/bmj.e1401.
- 4) Prevention of recurrent high-grade anal neoplasia with quadrivalent human papillomavirus vaccination of men who have sex with men: a nonconcurrent cohort study. Swedish KA, Factor SH, Goldstone SE. *Clin Infect Dis*. 2012 Apr;54(7):891-8.
- 5) Prevention of anal condyloma with quadrivalent human papillomavirus vaccination of older men who have sex with men. Swedish KA, Goldstone SE. *PLoS One* 2014 Apr 8;9(4):e93393. doi: 10.1371/journal.pone.0093393. eCollection 2014.

OC 04-07

VALUE OF PARTIAL HPV GENOTYPING IN THE FOLLOW-UP AFTER CONIZATION FOR CERVICAL DYSPLASIA

K. Friebe, P. Hillemanns, M. Jentschke

Department of Gynaecology and Obstetrics, Hannover Medical School, Hannover (Germany)

Background / Objectives

To assess the clinical usefulness of partial genotyping with a clinically validated multiplex high-risk human papillomavirus (hrHPV) DNA-based assay (Abbott RealTime High-Risk HPV assay (RealTime) versus composite detection of hrHPV DNA by Hybrid Capture 2 (hc2; Qiagen Hybrid Capture 2 High-Risk HPV DNA Test) in the follow-up after conization for cervical intraepithelial neoplasia (CIN).

Methods

A total of 148 women that underwent conization between January 2007 and December 2013 had a pre-operative examination and one follow-up visit after a mean interval of 6.4 months. Pre/post-treatment cytology and hrHPV DNA status, conus height, conus weight and resection margins and menopausal status were evaluated in this retrospective analysis. 105 women had been tested with hc2 targeting 13 hrHPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 43 with RealTime targeting the same hrHPV types plus HPV66 with partial genotyping of HPV16 and HPV18. Correlation between recurrent CIN and pre- and post-operative hrHPV status and other cofactors was established. Residual disease or recurrence was defined as either histologically confirmed CIN 2+, suspicious colposcopy or cytology with high-grade squamous intraepithelial lesions or worse (HSIL+).

Results

Complete data sets were available for 144 women, of whom 21 (14.6%) had residual/recurrent disease at the first follow-up after conization. Conus height and weight and resection margins, as well as menopausal status were no significant predictors for residual/ recurrent disease.

Persistent HPV infection, detected by hc2 and RealTime, showed significance for predicting recurrence ($p < 0.001$ vs. $p < 0.01$) and had high sensitivity and specificity rates (hc2: 90.0% and 82.4%; RealTime: 63.6% and 85.7%). HPV16 persistence

identified with RealTime exhibited higher sensitivity and specificity (83.3% and 100%) than detection of non-HPV16/18 genotypes (28.6% and 87.5%) and was, moreover, most significant for indicating recurrence of CIN ($p < 0.001$ vs non-HPV16/18 $p = 0.557$). Cotesting with hc2 and cytology, RealTime and cytology, as well as HPV16 and cytology were highly significant ($p < 0.001$) for the detection of recurrent/residual CIN. The highest sensitivity and specificity for the detection of recurrent/residual disease were achieved with cotesting of HPV16 and cytology (100% and 100%; hc2 and cytology: 90% and 82.8%; RealTime and cytology: 100% and 86.7%).

Conclusion

In this retrospective analysis the highest sensitivity and specificity for recurrent/residual disease in the follow-up after conization for cervical dysplasia was achieved with detection of HPV16 and cotesting of HPV16 and cytology.

OC 04-08

PERFORMANCE OF P16/KI-67 DUAL-STAINED CYTOLOGY FOR MONITORING WOMEN TREATED FOR HIGH-GRADE CIN

N.J. Polman¹, **M.H. Uijterwaal**¹, **B.I. Witte**², **J. Berkhof**², **D.A.M. Heideman**¹, **R.D.M. Steenbergen**¹, **F.J. Van Kemenade**³, **J.W.M. Spruijt**⁴, **M.W. Van Baal**⁵, **G.C.M. Graziosi**⁶, **D.K.E. Van Dijken**⁷, **R.H.M. Verheijen**⁸, **T.J.M. Helmerhorst**⁹, **R. Ridder**¹⁰, **P.J.F. Snijders**¹, **C.J.L. Meijer**¹

¹Department of Pathology, VU University Medical Center, Amsterdam (Netherlands), ²Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam (Netherlands), ³Department of Pathology, Erasmus University Medical Center, Rotterdam (Netherlands), ⁴Department of Obstetrics and Gynecology, VU University Medical Center, Amsterdam (Netherlands), ⁵Department of Obstetrics and Gynecology, Flevo Hospital, Almere (Netherlands), ⁶Department of Obstetrics and Gynecology, Sint Antonius Hospital, Nieuwegein (Netherlands), ⁷Department of Obstetrics and Gynecology, Onze Lieve Vrouwen Gasthuis West, Amsterdam (Netherlands), ⁸Department of Obstetrics and Gynecology, University Medical Center Utrecht, Utrecht (Netherlands), ⁹Department of Obstetrics and Gynecology, Erasmus Medical Center, Rotterdam (Netherlands), ¹⁰Roche mtm laboraties AG, Mannheim (Germany)

Background / Objectives

Women treated for high-grade cervical intraepithelial neoplasia (CIN) are at risk of recurrent CIN (rCIN). Currently, women treated for CIN grade 2 or 3 (CIN2/3) are monitored by cytology or cytology/high-risk (hr)HPV co-testing. This study aimed to evaluate the performance of p16/Ki-67 dual-stained cytology for post-treatment monitoring.

Methods

325 of 364 women treated for high-grade CIN (SIMONATH-cohort) were included in this post hoc-study. Liquid-based cytology (LBC) samples collected at 6 and 12 months after treatment were tested for cytology, hrHPV and methylation markers. In case of any abnormal test result at 6 months women were referred for colposcopy-directed biopsy. Exit-colposcopy with biopsy was performed at 12 months follow-up. LBC samples obtained at, or close before, biopsy were retrospectively stained for

p16/Ki-67. Performance of cytology, hrHPV and p16/Ki-67 for detection of rCIN3 and rCIN2+ was compared.

Results

p16/Ki-67 showed a similar sensitivity for rCIN3 (84.6%; 95% CI: 54.6-98.1%) as cytology (92.3%; 95% CI: 64.0-99.8%) and hrHPV (100.0%; 95%CI: 75.3-100.0%), but with a significantly higher specificity (85.8%; 95% CI: 81.7-89.8%) compared to both cytology (66.7%; 95% CI: 61.2-72.1%) and hrHPV (71.5%; 95% CI: 66.3-76.6%). When combining p16/Ki-67 and hrHPV testing, sensitivity for rCIN2+ was comparable to sensitivity of cytology/hrHPV co-testing (85.0%; 95% CI: 73.9-96.1% vs. 87.5%; 95% CI: 77.3-97.7%), but with a significantly higher specificity (74.3%; 95% CI: 69.0-79.6% vs. 58.2%; 95% CI: 52.3-64.2%).

Conclusion

In post treatment monitoring p16/Ki-67 dual-staining tended to have slightly lower sensitivities for rCIN2/3, but significantly higher specificities compared to cytology and hrHPV testing. Combining p16/Ki-67 with hrHPV testing results in similar sensitivity, but significantly higher specificity compared to cytology/hrHPV co-testing. Therefore, p16/Ki-67 dual-stained cytology, especially when combined with hrHPV testing, is the marker of choice for post-treatment monitoring.

OC 04-09

ABLATION TECHNIQUES ADAPTED FOR LOW- AND MIDDLE- INCOME COUNTRIES

M. Cremer¹, J. Felix², L. Taxa³, M. Maza⁴, P. Castle⁵, M. Alvarez³, J. Gage⁶, J. Jeronimo⁷

¹Cleveland Clinic Lerner School of Medicine, Cleveland, Ohio (United States of America), ²University of Southern California Keck School of Medicine, Los Angeles, California (United States of America), ³INEN, Lima (Peru), ⁴Basic Health International, San Salvador (El Salvador), ⁵Albert Einstein College of Medicine, Bronx, New York (United States of America), ⁶National Cancer Institute, Rockville, Maryland (United States of America), ⁷PATH, Seattle, Washington (United States of America)

Background / Objectives

Cervical cancer is a leading cause of cancer-related mortality among women in low- and middle-income countries (LMICs). Two point-of-care technologies that address the treatment gap are the LMIC-adapted CryoPen®, with a core temperature of approximately -95°C, and the thermocoagulator, with a probe temperature of 100-120°C. Since there is scant data on the extent of CIN involvement in an under-screened population, determining mean cervical intraepithelial neoplasia (CIN) depth in an under-screened population will establish the depth of necrosis (DON) that ablative techniques need to achieve. The study aimed to establish the maximum depth of involvement of CIN3 and test whether the LMIC-adapted CryoPen® and thermocoagulator reach the DON established as necessary for eradicating CIN3.

Methods

A convenience sample of 107 CIN3 cases were reviewed by a pathologist at the National Cancer Institute (INEN, Peru) and a U.S. pathologist. Mean depth of involvement was measured in the CIN3 cases and mean DON was measured in the ablated cervical specimens. Ten women had ablative procedures before non-cervical pathology indicated hysterectomy: a single five-minute freeze with the CryoPen® (n=5) or a single 60-second, 100°C application of the thermocoagulator (n=5). Following the hysterectomy, the cervix was separated from the uterus and the anterior and posterior lips were separated and processed. The local pathologist, who was blinded to which ablative technique was used, measured maximum DON in both lips.

Results

Mean depth of CIN3 involvement was 2.0mm among 107 cases; 79.4% of cases had a mean depth ≤ 3.0 mm, 89.7% had a mean depth ≤ 3.5 mm, 93.5% had a mean depth ≤ 4.0 mm, and 6.5% had a mean depth ≥ 5.0 mm. The maximum DON achieved by the LMIC-adapted CryoPen® was ≥ 3.0 in 80% of cases, ≥ 3.5 mm in 80%, ≥ 4.0 mm in 80%, ≥ 4.5 mm in 40%, and ≥ 5.0 mm in 20%. The maximum DON achieved by the thermocoagulator was ≥ 3.0 mm in 80% of cases, ≥ 3.5 mm in 80%, ≥ 4.0 mm in 20%, and ≥ 4.5 mm in 20%. The CryoPen® achieved a mean DON of 4.12mm in the anterior lip (range 1.5 - 4.5mm) and 4.08mm in the posterior lip (range 2.8 - 6.0mm). The thermocoagulator achieved a mean DON of 3.54mm in the anterior lip (range 2.2 - 4.5mm) and 3.02mm in the posterior lip (2.0 - 4.1mm).

Conclusion

The pathology review of CIN3 cases showed that 90% of CIN3 would be eradicated if DON reached at least 3.5mm. The mean DON of both the LMIC-adapted CryoPen® and thermocoagulator exceeded 3.5mm. Attempts to improve the range of depths and overall efficacy will be emphasized through ongoing refinement of the devices and remedial training of clinicians providing the treatments.

OC 04-10

THE ACCURACY OF SPECIMEN DIMENSION IN DETERMINING VOLUME: PRETERM LABOUR RELATED RISK ASSESSMENT

X. Carcopino¹, J. Mancini², J. Gondry³, L. Préaubert¹

¹Department of Obstetrics and Gynaecology, Hôpital Nord, Assistance Publique des Hôpitaux de Marseille (APHM), Aix-Marseille Université (AMU), CNRS, IRD, Avignon Université, IMBE UMR 7263, 13397, Marseille, France (France), ²Aix Marseille Université, Inserm, IRD, UMR S912, SESSTIM, Marseille, 13385 France, APHM, Hôpital de la Timone, Département de santé publique, BioSTIC, Marseille, 13385 France (France), ³University Hospital of Amiens, Jules Verne University, Picardie, France (France)

Background / Objectives

Excision therapy was shown to be associated with a significant increased risk of premature delivery. Although recent evidence suggests that this risk mostly depends on the volume of the excised specimen. such hypothesis results from studies having retrospectively calculated the excised volume from specimens' dimensions and not on genuine prospective measurement of the excised volume. The aim of this work was to estimate whether the calculation of the excised volume from its dimensions is reliable.

Methods

A multicentric prospective observational study was conducted in 9 French hospitals: 258 patients who had a LLETZ performed for the therapy of CIN2-3 were included. The exact dimensions (thickness. length and circumference) and the volume of the specimen were measured at the time of procedure. before formaldehyde fixation. The excised volume was measured by immersing the specimen in a 25 ml graduated tube filled with water using the fluid displacement technique. The measured volume was then compared to the calculated volume using volume formulas of a cone. a cylinder. a parallelepiped and a semi sphere as previously reported in literature.

Results

The mean thickness. length and circumference of excised specimens were 8.8 (± 3.8). 12.7 (± 5.9) and 45.7 (± 16.8) mm, respectively. The mean measured volume was 2.53 (± 1.49) ml. Using the formula for the volume of a cone, a cylinder, a parallelepiped and a semi sphere, estimated volumes were 0.92 (± 1.15). 4.18

(± 6.76). 6.20 (± 7.31) et 2.06 (± 2.4) mL, respectively. The highest intraclass correlation coefficient between measured and calculated volume was observed when using the formula for the volume of a semi sphere: 0,46 (95%CI: 0,36-0,56). Two previous retrospective studies estimated the risk of premature delivery to be significantly increased if the excised volume exceeds 6 and 2.65 mL, respectively (1, 2). Based on such previous results and according to our results, the threshold value for the excised volume resulting in a significantly increased risk of premature delivery is 3 ml.

Conclusion

Using specimen dimensions, the formula for the volume of a semi sphere is the best predictor of the excised volume. Other formulas do not allow for a reliable estimation of the excised volume. Based on current literature, the risk of premature delivery is likely to be significantly increased if the excised volume exceeds 3 ml.

References

- 1.Khalid S, Dimitriou E, Conroy R, Paraskevoidis E, Kyrgiou M, Harrity C, Arbyn M, Prendiville W. The thickness and volume of LLETZ can predict the relative risk of pregnancy-related morbidity. *BJOG*. 2012;119(6):685-91.
- 2.Castanon A, Landy R, Brocklehurst P, Evans H, Peebles D, Singh N, Walker P, Patnick J, Sasieni P; PaCT Study Group. Risk of preterm delivery with increasing depth of excision for cervical intraepithelial neoplasia in England: nested cas-control study. *BMJ*. 2014 Nov 5;349:g6223.

OC 04-11

THE INCREASED DETECTION OF CIN2+ BY ZEDSCAN (EIS) IS INDEPENDENT OF HR-HPV GENOTYPE

J. Tidy ¹, B. Brown ², J. Healey ³, R. Lyon ⁴, J. Palmer ⁵

¹Gynaecological Oncology, Sheffield Teaching Hospital NHS Foundation Trust, Sheffield (United kingdom), ²Medical Physics, University of Sheffield, Sheffield (United kingdom), ³Medical Physics, Sheffield Teaching Hospital NHS Foundation Trust, Sheffield (United kingdom), ⁴Colposcopy Clinic, Sheffield Teaching Hospital NHS Foundation Trust, Sheffield (United kingdom), ⁵Gynaecological Oncology, Sheffield Teaching Hospital NHS Foundation Trust, Sheffield (United kingdom)

Background / Objectives

To study the impact of hrHPV genotype on performance of ZedScan (Electrical Impedance Spectroscopy) with colposcopy and colposcopy alone in detection of CIN2+ within a routine colposcopy service. Some studies have suggested that CIN associated with hrHPVs other than HPV16 may be associated with less aceto-white change and smaller in size hence more difficult to detect ¹⁻³.

Methods

1564 unselected women were evaluated by six colposcopists, three nurse colposcopists two consultants and a trainee. 93% of the women were evaluated by nurse colposcopists. HPV genotyping was performed using Roche COBAS 4800. Results are given as HPV16, HPV18 and HPV Other (O) All data were collected prospectively. Fisher's exact test, two tailed, was used for statistical analysis.

Results

1229 women were referred with abnormal cytology, 335 had other indications which included 228 women referred after a positive hrHPV/cytology negative result. 396 (25.3%) had high grade cytology, 833 (53.3%) had low grade cytology. 500 women were found to have CIN2+, 54 of these women were identified as having CIN2+ by ZedScan alone resulting in a 12.1% increase in the detection of CIN2+ ($p < 0.0001$). In women referred with low grade cytology the detection of CIN2+ increased by 44.9%. HPV genotyping was available for 788 (50.4%) women; 569 abnormal cytology, 181 hrHPV positive/cytology negative and 36 other indications. Single infection with HPV16 was found in 151 cases, HPV18 51 cases and HPV O 422 cases, there were 164 cases of multiple HPV infection. The addition of ZedScan increased the detection of CIN2+ from 88.7 to 95.2% in women with HPV16 associated infections

(either single or multiple) ($p=0.1006$) and in women with HPV18 or HPV O infections there was a significant increase from 79.5% to 95.9% ($p=0.0001$).

Conclusion

Performance of ZedScan with colposcopy significantly exceeds those of colposcopy alone. There was a significant increase in the detection of CIN2+ by ZedScan in women with non HPV16 infections. The detection of CIN2+ by ZedScan is independent of HPV genotype.

References

1. Jeronimo J, Massad S, Schiffman M, et al. Visual appearance of the uterine cervix: correlation with human papillomavirus detection and type. *Am J Obstet Gynecol* 2007;197:47.e1-47.e8.
2. Nam K, Kim JK, Jeon S. Human Papillomavirus Type 16 Causes Larger Colposcopic Lesions Than Other HPV Types in Patients With Grade 3 Cervical Intraepithelial Neoplasia *Journal of Lower Genital Tract Disease*, Volume 17, Number 1, 2013, 1-5
3. van der Marel J, van Baars R, Quint WGV, Berkhof J, del Pino M, Torne A, Ordi J, Wentzensen N, Schiffman M, van de Sandt, MM, Lindeman J, Jenkins D, Helmerhorst TJM, Verheijen RHM, ter Harmsel B, Alonso I. The impact of human papillomavirus genotype on colposcopic appearance: a cross-sectional analysis. *BJOG* 2014;121:1117–1126.

OC 04-12

RISK FOR PREMATURE BIRTH AFTER EXCISION OF CERVICAL INTRAEPITHELIAL NEOPLASIA: A POPULATION-BASED STUDY OF SINGLETON PREGANANCIES

N. Jancar¹, B. Mihevc Ponikvar², S. Tomsic²

¹Department of Human Reproduction, Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šljmerjeva 3, Ljubljana (Slovenia),

²Health Survey and Health Promotion Department, National Institute of Public Health, Trubarjeva 2, Ljubljana (Slovenia)

Background / Objectives

Cervical intraepithelial neoplasia, a potential precursor for cervical cancer, is caused by persistent Human Papillomavirus infection. These lesions are most prevalent in women in their childbearing age. Screening programs for cervical cancer are implemented in many countries, including Slovenia, and the incidence of cervical cancer has fallen substantially in these countries. As a result, more and more low- and high-grade cervical lesions are detected every year due to growing population of women attending organized screening. It has been found, that treatment of cervical neoplasia with excision methods is an important risk factor for premature birth.

The aim of our study was to explore the association between cold-knife conisation and large loop excision of transformation zone (LLETZ) and preterm birth in a 10-year national sample.

Methods

For our retrospective population-based study we used data from Medical Birth Registry – the National Perinatal Information System of Slovenia. We included all singleton births in Slovenia in the period from January 1st 2003 to December 31st 2012. A medical history of cold-knife conisation or LLETZ served as exposure variables for the calculation of the odds ratio (OR) for preterm birth according to gestational age of the newborn. Logistic regression analyses were used to evaluate the association between cervical excision procedures and preterm birth.

Results

There were 195.468 singleton births in our study period. A total of 2.127 (1.1%) women had a history of cold-knife conisation and 2.542 (1.3%) women had a history of LLETZ. A total of 903 singletons (0.5%) were born with the gestation less than 28 weeks, 1111 singletons (0.6%) with the gestation 28 to 31 weeks, 1310 singletons (0.7%) with the gestation 32 to 33 weeks and 7834 singletons (4.0%) with the gestation 34 to 36 weeks. The OR (and 95% confidence interval) for extreme preterm birth, before 28 weeks, was 5.1 (3.76 – 6.85) after cold-knife conisation and 2.7 (1.90 – 3.96) after LLETZ. The OR for very preterm birth, before 32 weeks, was 4.8 (3.90 – 5.93) and 2.4 (1.86 – 3.24), respectively. The OR for preterm birth before 34 weeks was 4.3 (3.63 – 5.14) and 2.4 (1.96 – 2.97), respectively. The OR for preterm birth before 37 weeks was 2.9 (2.58 – 3.29) and 1.8 (1.61 – 2.10), respectively. All OR had a p value < 0.001.

Conclusion

Women with a history of cervical excision procedure (cold-knife conisation or LLETZ) have significantly increased odds for preterm birth, especially for extremely and very preterm births.

OC 04-13

SEVERE FORMS OF CONDILOMATA (GENITAL WARTS) IN THE GENITOANAL REGION THE NEW RADIO WAVE TECHNIQUE AS THE MOST EFFECTIVE THERAPEUTIC AND ETHETIC SOLUTION

J. Igor

Ordination Jeremic (Serbia)

Background / Objectives

Condiloma epidemic in the recent time as the consequence of low risk HPV type 6 and 11 infection , affecting primarily younger generation , girls from 18 to 23 years of age.High incidence of HPV infection , accompanying by condilomata is the result of very easy transmittance since it is a sexually transferred illness.STD.Only one unprotected intercourse (without protection of condom) is sufficient since it is sexually transmitted disease.The awareness of changes in the genital and anus area with young girls is a cause of fear and concern.This calls for additional responsibility in diagnostic approach and choice of best therapy.

Methods

The study has been conducted on 150 patients from 16 to 50 years of age, both sexes, including pregnant women and immune deficient patients with medium to heavy forms of condilomata in the entire region of genitals, anus and intraanus in mens.This all present a huge therapeutic challenge on account of the following:

- 1.sensibility of the genitals and anus area to forced trauma
- 2.inaccessibility of approach
3. high vascularization of vagina,cervix and hemorrhoid circle area.
- 4.exposure to infection , bacteria flora (vagina and colon)

Results

New technique of radio waves of 4 MHZ combines in a special way excision and vaporization , which result in almost bloodless work area, and ensures precision in total removal of condilomata in one medical procedure , and in severe cases,20% with very heavy and some of Buschke Loewenstien forms , two procedures in local anesthesia.The time for the intervention is from 5 to 30 minutes without

hospitalization. Thanks to new procedure technique and specially designed attachments, the lateral damage to healthy tissue is less than 10 microns. Insignificant lateral damage to the tissue and minimum bleeding do not affect the local immunity which is key to speedy recovery without infection and relapses percentage of less than 3% in the follow up period from 3 to 18 months. With young girls, the absence of esthetic status is paramount since there are no scars and traces of intervention on the treated area, and the functionality of the same is not affected at all.

Conclusion

RW 4 Mhz system and described technique is a new, effective, painless, safe and bloodless method with maximum therapeutic and esthetic effect and the least known rate of relapses, below 3% with these forms of condylomata. The ease of technique in local anesthesia makes it a therapy of choice even during pregnancy. It takes approximately 5 weeks of total recovery and abstention after intervention from sexual intercourse, regardless of the gravity of case in question.

References

Patented the special technique of radiowave elimination of the worst forms of anal and intra-anal condylomas, including giant condylomata (Busche-Lowenstein) of other locations in both sexes. Presented on numerous world congresses. Case studies are also presented on Medical Faculties in the USA as examples of treatment of choice.

. Patented the special technique of radiowave LOOP excision with radiowave vaporization as a method of treatment of choice with difficult cases of dysplasia on PVU (CIN I, CIN II, CIN III)

Patented the special technique of removal of condyloms on vagina and mucous membrane of labia (radiowave vaporization)

. Patented the technique of removal of giant anal and intraanal condyloma, with the use of the mixed technique of radiowave vaporization and excision, without bleeding.

OC 05-01

HIGH-RISK HPV DETECTION IN PLASMA SAMPLES OF WOMEN WITH A RECENT HISTORY OF CERVICAL DYSPLASIA.

M. Martinelli ¹, R. Musumeci ¹, E. Calaresu ¹, E. Tanzi ², G. Sotgiu ³, A. Piana ³, C. Cocuzza ¹

¹Department of Medicine and Surgery, University of Milano-Bicocca (Italy),

²Department of Biomedical Sciences for Health, University of Milano (Italy),

³Department of Biomedical Sciences, University of Sassari (Italy)

Background / Objectives

Circulating HPV DNA has been previously described in women with advanced stages of cervical cancer and has been suggested to be a prognostic marker of disease recurrences and metastases^{1,2}. The presence of HPV in plasma of women with early stages of cervical dysplasia has not been previously reported. The aim of this study was to investigate the presence of high-risk HPV (hrHPV) in cervical and plasma samples of women with a recent history of cervical dysplasia.

Methods

Blood and cervical samples were obtained from 120 women referred to San Gerardo Hospital, Monza, Italy with a recent history of ASCUS or low-grade cervical dysplasia (L-SIL). Routine cervical cytology (Pap test) and hrHPV detection from cervical and plasma samples were performed on all samples. A control group of 20 healthy females was included for hrHPV testing in plasma. Nucleic acid extraction was performed using automated NucliSENS easyMAG system (bioMérieux). HPV 16, 18, 31, 33, 45, 51 and 52 detection was carried out by means of previously described genotype-specific "in house" real-time PCR assays^{3,4,5} and confirmed by Anyplex II HPV28 (Seegene); all assays were validated through the participation the Laboratory to the WHO LabNet HPV Proficiency Study.

Results

At the time of blood collection, cervical cytology results showed 26 women to have regressed to a negative Pap test, 24 with ASCUS, 52 L-SIL and 18 H-SIL.

Overall hrHPV positivity for one or more of the tested high-risk genotypes showed rates of 44.2% (53/120) in cervical samples, 34.2% (41/120) in plasma, and 20.8% (25/120) of women were found to have hrHPV in both blood and cervix.

In particular, plasma HPV DNA positivity rates according to cervical cytology results at the time of blood sample collection were: negative cytology 30.8% (8/26); ASCUS 33.3% (8/24); L-SIL 30.7% (16/52); H-SIL 50% (9/18). No HPV positivity was found in blood samples of women from the control group.

HPV 45 (46.2%), 51 (30.8%) and 16 (17.3%) were found to be the most prevalent genotypes in positive plasma samples whilst HPV 31 and 33 were not detected.

Conclusion

In this study, real-time PCR detected HPV 45, 51, 16, 52 and 18 DNA in the peripheral blood of women with a recent history of ASCUS or low-grade cervical dysplasia, with positivity rates ranging from 30.7% to 50% depending on cervical cytology results at the time of blood collection. This is to our knowledge the first report of hrHPV DNA detection in plasma samples of women with an early history of cervical dysplasia. Further studies are required to evaluate the significance of hrHPV DNA detection in the circulatory system of women with transient or early stages of cervical dysplasia.

References

1. Sathish N et al. J Clin Virol 2004; 31:204-9
2. Wei Y C et al. Int J Gynaecol Obstet 2007; 96:112-6
3. Broccolo F, Cocuzza C. J Med Virol 2009; 81:278-287
4. Lindh M et al. J Clin Virol 2007; 40:321-324
5. Flores-Munguia R et al. J Mol Diagn 2004; 6; 2:115-124

OC 05-02

AEROBIC VAGINITIS, CONTRARILY TO BACTERIAL VAGINOSIS, IS A RISK FACTOR FOR MAJOR PAP SMEAR ABNORMALITIES

P. Vieira-Baptista¹, J. Lima-Silva¹, C. Pinto¹, C. Saldanha², J. Beires¹, G. Donders³

¹Serviço de Ginecologia e Obstetrícia, Centro Hospitalar de São João, Porto (Portugal), ²LAP—Laboratório de Anatomia Patológica, Unilabs, Porto (Portugal), ³Department of Obstetrics and Gynecology, University Hospital Antwerp, Antwerp; Femicare Clinical Research for Women, Tienen (Belgium)

Background / Objectives

Bacterial vaginosis (BV) has been associated with human papilloma virus infection (HPV) and abnormal Pap smears. However, most studies neglect the existence of another entity, which sometimes is confused with BV: aerobic vaginitis (AV). This study intended to evaluate the relation between major Pap smear abnormalities (MPSA), HPV infection and AV or BV.

Methods

Cross sectional study, conducted between June 2014 and February 2016, consisting of the systematic collection of vaginal discharge to a slide, previously to the Pap smear. The slides were evaluated blindly by one of the authors, according to Donders' criteria. All Pap smears were read at the institution's laboratory; cobas® HPV (Roche©) test was performed in most smears.

Results

Out of 959 cases, 832 (86,8%) were enrolled at the Cervical Pathology Unit of our institution. The mean age of patients was 41.4±10.92 years.

There were 13.6% (130/959) of cases of Pap smear worse than LSIL and 46.1% (436/946) were high risk (HR) HPV positive. The prevalence of BV was 15.5% (149/959) and that of moderate or severe AV was 7.4% (71/959). Women with BV had a relative risk (RR) of having a MPSA of 1.36 (IC 95% 0.92-2.01) while in those with AV the RR was 1.76 (IC 95% 1.10-2.79). If AV and BV were considered together (abnormal vaginal flora, AVF) the RR was 1.62 (IC 95% 1.16-2.27).

Neither AV nor BV were significantly associated with HR HPV infection as such (43.7% [31/71] vs. 54.7% [479/875], $p=0.083$ and 52.7% [78/148] vs. 54.1% [432/798], $p=0.788$, respectively). Stratifying for the genotypes (16, 18 and other HR HPV) there were also no differences.

Conclusion

AV, but not BV, was associated with major Pap smear abnormalities. However, if AV is misclassified as BV, an association still can be found between AVF and Pap smear abnormalities. Neither AV nor BV were associated with HPV infection, in the absence of cervical lesions.

OC 05-03

EPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS TYPE 67 IN BELGIAN WOMEN

S. Nouws¹, A. Carregosa², I. Benoy¹, J. Bogers³, D. Vanden Broeck¹

¹AML, Department of Molecular Diagnostics, Antwerp, Belgium (Belgium),

²University of Lisbon, Instituto Superior Técnico, Lisbon, Portugal (Portugal),

³University of Antwerp, AMBIOR, Antwerp, Belgium (Belgium)

Background / Objectives

Human papillomaviruses (HPV) possess the ability to induce cervical intraepithelial neoplasia. Fourteen HPV genotypes are classified by IARC as being group 1 carcinogens, or high-risk HPV types. It was noted that other HPV types were identified as single HPV infections in certain rare cases of cervical cancer (HPV26, 53, 67, 70, 73, 82), termed potential high-risk HPV types. In general, HPV67 has only been limited subject of research. Therefore, the objective of this study was to explore the epidemiology of HPV67 in a general screening and diagnostic population of Belgian women. Furthermore, cyto-virological correlation between HPV67 and the different cytological categories was explored.

Methods

Samples were analyzed using a high-throughput type-specific TaqMan-based real-time quantitative PCR. In total, 478.822 samples were evaluated between 2009 and 2015 for presence of HPV67. There were 375.988 samples collected from a preventive screening population and 102.834 samples from women in a diagnostic follow up.

Results

Overall, HPV67 was found in 1,85% of the examined samples. A significant higher prevalence was found in the diagnostic population (4,11%) compared to the screening population (1,22%). In 48,64% of the HPV67 infected women from the preventive group, it was detected as a single infection. In the diagnostic population, the proportion of HPV67 single infections was 35,17%. According to cytological categories, HPV67 was found in 0,75% of Negative for Intraepithelial Lesion (NILM); 6,34% of Atypical Squamous Cells of Undetermined Significance (ASC-US); 7,81% of Low-grade Squamous Intraepithelial Lesion (LSIL); 5,71% of ASC-cannot exclude HSIL (ASC-H); 5,60% of High-grade SIL (HSIL) and 9,09% (1/11 cases) of cancer cases in the screening population. In the diagnostic group 2,66% of NILM; 6,90% of ASC-US; 7,66% of LSIL; 5,12% of ASC-H; 4,91% of HSIL and no cancers cases

were found to be HPV67 positive. During microscopic examination, 1.034 samples were diagnosed as Atypical Glandular Cells (AGC). Within this category, HPV 67 appeared to be present in 1,84%. In more than half of these cases, HPV67 was found to be a single infection (57,89%). This phenomenon was discovered to be more pronounced in the screening (71,43%) versus the diagnostic (50,00%) population. The only HPV67 positive cancer case was categorized as a cervical adenocarcinoma, albeit in combination with other HPV genotypes.

Conclusion

The non-negligible prevalence of HPV67 indicates that further investigation and study on epidemiology is required for the understanding of its role in the genesis of cervical cancer.

References

1. Bouvard, V., et al., A review of human carcinogens--Part B: biological agents. *Lancet Oncol*, 2009. 10(4): p. 321-2.)
2. Schiffman, M., G. Clifford, and F.M. Buonaguro, Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer*, 2009. 4: p. 8.)
3. Geraets, D., et al., Detection of rare and possibly carcinogenic human papillomavirus genotypes as single infections in invasive cervical cancer. *J Pathol*, 2012. 228(4): p. 534-43.)

OC 05-04

UNMET MEDICAL NEEDS FOR HUMAN PAPILLOMAVIRUS INFECTION AND DIAGNOSIS

F. Alhamlan

King Faisal Specialist Hospital and Research Centre (Saudi Arabia)

Background / Objectives

Human papillomavirus (HPV) infection has been considered as a significant etiological factor and an important prognosticator in cervical cancer. Indeed, researchers have confirmed the role of high-risk human papillomaviruses in over 70% of cervical cancer cases. Accurate molecular diagnostic techniques for HPV detection and identification are of great importance for determining and diagnosing at-risk patients. However, the medical field still lacks standardized detection/genotyping assays. Herein, we present our research on developing and validating detection and genotyping assays of HPV. We also have determined the prevalence, sociodemographic characteristics and sexual behavior as risk factors for human papillomavirus (HPV) infection in a hospital-based cohort of women in Saudi Arabia.

Methods

We have collected cervical specimens and questionnaire data from 1200 women attending clinics in Riyadh, Saudi Arabia. Cervical specimens were examined for abnormal cytology using a standard Pap test and for the presence of HPV-DNA using reverse line blot hybridization, Luminx and GenoFlow tests.

Results

We have found that approximately 7% of the women were HPV-positive, 73% of them were Saudi nationals. Nearly 50% were under 40 years old (range, 22–80 years; mean \pm SD, 41.20 \pm 10.43 years). The most commonly detected HPV types were HPV-18 (34%) and HPV-16 (19%), with multiple infections detected in 10% of positive specimens. Multivariate analyses revealed that smoking and multiple partners were significant risk factors for HPV infection ($P < 0.01$).

Conclusion

Because of societal challenges and an unsubstantiated assumption of low HPV prevalence, few studies have examined sociodemographic characteristics or sexual behaviors associated with HPV in Saudi women. Our project will contribute greatly to

the global knowledge about HPV prevalence in Saudi Arabia as well as standardization of HPV detection and genotyping assays.

OC 05-05

IMPACT OF HPV 16, 18 AND OTHER HR-HPV TYPES ON INVASIVE CERVICAL CANCER SURVIVAL IN BRAZIL

M.L.N.D. Genta¹, J.P. Carvalho¹, R.M. Lopez¹, T.R. Martins², J.E. Levi²

¹Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina, São Paulo, São Paulo, Brazil. (Brazil), (Brazil), ²Laboratório de Virologia, Instituto de Medicina Tropical, Universidade de São Paulo, São Paulo, São Paulo, Brazil (Brazil). (Brazil)

Background / Objectives

Invasive cervical cancer is the second most common malignant tumor affecting Brazilian women. The distribution of Human Papillomavirus (HPV) genotypes in Brazil has not been extensively studied. Little is known about the impact of HPV genotypes on invasive cervical cancer survival.

Methods

Women with a diagnosis of invasive cervical cancer between November 2008 and July 2012 were recruited from the Instituto do Câncer do Estado de São Paulo (ICESP). DNA was extracted from three 10µM-thick paraffin-embedded cervical carcinoma tissue per subject using the automated BD Viper LT System and high-risk HPV detection and genotyping was determined using the BD Onclarity HPV Assay. Age at diagnosis, clinical staging, histological type and survival data were obtained from the hospital's electronic data records.

Results

One hundred-ninety-six women with a median age of 51±14 years (range=17-87 years) were studied. Squamous cell carcinoma and adenocarcinoma were diagnosed in 80 and 20% of patients, respectively. Regarding clinical staging (FIGO), 31% were classified as 1A1 to 1B1, 17% as 1B2 to 2A and 52% as 2B to 4A.

The most frequent types were HPV16 (62 %), HPV18 (9%), HPV45 (5%), HPV31 (4%), HPV52 (2%), and others high risk HPV type (18%). Most infections (72%) were caused by individual HPV types, with 28% harboring 2 or more co-infecting types.

There were 66 deaths during up to 118 months of follow-up. Kaplan-Meier survival curves and log-rank statistics revealed that HPV 16/18 (n= 147) did not have a worse prognosis as compared to other HPV types (n=49) (P=0.603). Age at diagnosis,

clinical staging, histological type and HPV type were included in a Cox regression model. Only clinical staging was independently associated with survival.

Conclusion

To our knowledge, this is one of the largest studies that assessed HPV genotype from invasive cervical cancer paraffin-embedded tissue samples in Brazil. The present study confirms the continuing major role of HPV16 and 18 in invasive cervical cancer and is similar to other country findings. The observation that more than $\frac{1}{4}$ of all cancers are caused by non-16/18 types with similar prognosis underlines the importance of extended genotyping in disease detection. Clinical staging at diagnosis was the only predictor of survival, reinforcing the importance of cervical cancer screening and diagnosis at early stages.

References

- 1.Cuschieri K, Brewster DH, Graham C, Nicoll S, Williams AR, Murray GI, et al. Influence of HPV type on prognosis in patients diagnosed with invasive cervical cancer. *Int J Cancer*. 2014;135(11):2721-6.
- 2.Amaro-Filho SM, Golub JE, Nuovo GJ, Cunha CB, Levi JE, Villa LL, et al. A comparative analysis of clinical and molecular factors with the stage of cervical cancer in a Brazilian cohort. *PLoS One*. 2013;8(3):e57810.
- 3.de Oliveira CM, Fregnani JH, Carvalho JP, Longatto-Filho A, Levi JE. Human papillomavirus genotypes distribution in 175 invasive cervical cancer cases from Brazil. *BMC cancer*. 2013;13:357.
- 4.Bravo IG, Felez-Sanchez M. Papillomaviruses: Viral evolution, cancer and evolutionary medicine. *Evolution, medicine, and public health*. 2015;2015(1):32-51.
- 5.Darus CJ, Mueller JJ. Development and impact of human papillomavirus vaccines. *Clinical obstetrics and gynecology*. 2013;56(1):10-6.
- 6.Castellsagué X. Natural history and epidemiology of HPV infection and cervical cancer. *Gynecologic oncology*. 2008;110(3 Suppl 2):S4-7.

OC 05-06

INCIDENCE OF CONDYLOMA 7 YEARS POST-VACCINE AVAILABILITY

E. Herweijer, A. Ploner, L. Arnheim-Dahlström, P. Sparén

Karolinska Institute, Department of Medical Epidemiology and Biostatistics, Stockholm (Sweden)

Background / Objectives

HPV types 6 & 11, included in the quadrivalent HPV vaccine (qHPV), are found in the majority of the condyloma cases. Sweden has offered subsidized qHPV vaccination to 13-17 year old girls between 2007-2011 after which an organized school-based vaccination program for girls ages 10-12 and catchup for girls ages 13-18 begun. Due to the short incubation time of condyloma, the effect of vaccination can be studied shortly after qHPV introduction. The aim of this study was to assess the incidence of condyloma after the introduction of qHPV in Sweden.

Methods

Trends in condyloma incidence over time was studied with an ecological study design. The study population included all men and women at ages 10-44 during the study period 2006-2012. First occurring condyloma cases were identified via the Swedish Patient register and Prescribed Drug register, where pharmaceuticals prescribed for treatment against condyloma was used as a proxy. Mean population estimates by sex, age, and calendar year were obtained from Statistics Sweden. Poisson regression was used to model the incidence rates and the corresponding 95% confidence intervals (CI) of condyloma stratified for age. Average annual percent changes (AAPC) were estimated for the post-vaccine availability period 2007-2012 by means of a broken line regression model using a Poisson distribution.

Results

Decreases in incidence were observed in the post-vaccine availability period for women up to ages 34 with the greatest declines seen for girls ages 15-19 (AAPC=-11.1%, CI [-10.2;- 12.0]) and 20-24 (AAPC=-9.1%, CI [-8.3;- 9.8]) years old and with steeper declines in incidence from 2009 and onwards. Smaller reductions with no obvious breakpoints were seen for women ages 25-29 (AAPC=-7.6%, CI [-6.6;- 8.7]) and 30-34 (AAPC=-4.4%, CI [-2.9;-5.9]) years. Similar trends in incidence were seen in men, though smaller reductions in incidence were observed (AAPC ages 15-19=-3.9%, CI [-2.3;-5.4]; AAPC ages 20-24=-6.2%, CI [-5.4;-6.9]).

Conclusion

The observed decline in condyloma incidence in girls below age 25 following the introduction of qHPV in Sweden indicates anticipated effects. The observed decline among men and in women age 25 and above also points towards possible herd effects. Although an ecological study design cannot provide information on causality, the decline in condyloma among girls is well in line with observational studies comparing vaccinated with unvaccinated individuals. Future monitoring of the disease burden over time, as well as observational studies comparing vaccinated and unvaccinated individuals, is necessary to evaluate whether the qHPV program has the anticipated effect in the population.

OC 05-07

Prevalence of HPV types in a sample of women with abnormal cervical cytologies in Italy

D. Martinelli, F. Fortunato, M.G. Cappelli, R. Prato

Department of Medical and Surgical Sciences, University of Foggia (Italy)

Background / Objectives

In Italy, organized cervical screening programmes were started since 2001. In 2012, the target population amounted to 87.3% of women aged 25-64 years. Compliance to invitation was 40.8%, with a strong decreasing North-South trend. Currently, data on HPV types prevalence are available regarding healthy women undergoing spontaneous or organised screening. Little is known about the prevalence of type-specific HPV among women with LSIL, more severe dysplasia, or invasive cancer. A national HPV immunization programme in Italy was introduced in 2008 for females aged 11-12 years; in addition, some regions have extended the offer of vaccination to one or more older birth cohorts through 26 years.

Methods

We conducted a cross-sectional survey among a large number of women with LSIL, HSIL, ASC-H, ACG, squamous cell carcinoma, AIS, or adenocarcinoma recruited into the organized screening programme across Apulia region (1,130,000 women aged 25-64 years) between 2010-2012. We collected demographics characteristics, information on possible risk factors for HPV infection, and type-specific HPV-DNA testing. In order to assess the association between the HPV status of participants and other variables of interest a logistic regression model was performed.

Results

The overall prevalence of HPV infection was 86% (1,182/1,370 women with abnormal cytology tested for HPV, 95%CI=84%-88%); 78% (1,063/1,370, 95%CI=75%-80%) of these infections were identified as HR-HPV (83%, 95%CI=80%-86% among the youngest group 25-34 years) and 34% (460/1,370, 95%CI=31%-36%) were LR-HPV. The prevalence of coinfections with HR and LR types was 25% (349/1,370, 95%CI=23%-28%).

A total of 526 HPV genotypes, including 352 genotypes belonging to the "12 HR-HPV IARC group 1", were found in 294 women. The overall prevalence of HR-HPV was 81% (238/294, 95%CI=76%-85%), 87% (95%CI=80%-92%) among 25-34 year olds.

The proportion of the HR vaccine genotypes targeted by both the bivalent and quadrivalent vaccines was 53% (187/352); the proportion of HR-HPV genotypes covered by the nonavalent vaccine was 78% (275/352). Cross-protection against non-vaccine HR-HPV closely related to HPV16/18 was 67% (237/352) for the bivalent and 63% (222/352) for the quadrivalent vaccine.

HPV infection was more common among women single (OR=2.9, 95%CI=1.4-6.1, $p<0,05$), divorced (OR=2.7, 95%CI=1.2-6.2, $p<0,05$), holding a bachelor's degree (OR=2.8, 95%CI=1-7.9, $p<0,05$), and living in metropolitan areas (OR=5.3, 95%CI=1.4-6.1, $p<0,05$).

Conclusion

Our findings contribute to anticipate the full impact of HPV vaccines on the burden of cervical disease including cancer and its current and future effects on population based cervical screening programmes.

OC 05-08

PREVALENCE AND DISTRIBUTION OF HUMAN PAPILLOMAVIRUS TYPES AMONG THAI HILL TRIBE FEMALES IN RURAL AREA, NAN PROVINCE, THAILAND

N. Kantathavorn, U. Chatchotikawong, P. Sattayarungsee, C. Taepisitpong, S. Saeloo, W. Krongthong, W. Krisorakun, T. Sricharunrat, N. Teerayathanakul, S. Petchjorm, J. Mungdee, P. Krajokkaew

Chulabhorn Hospital (Thailand)

Background / Objectives

Following the wide diversity of population, ranging from Bangkok metropolitan women to hill tribe females, Thailand is considering future use of HPV primary screening, especially in the country's northern rural areas. With high and sophisticated mountains and villages of Nan province, most females in this area were poor and lack of opportunity to achieve high technology of medical services. Whilst, there are currently unavailable and very limited data on HPV infection among Thai hill tribe women. This study aims to determine the prevalence and distribution of HPV types among Thai hill tribe females living in Tha Wang Pha, Pua, and Bo Kluea districts of Nan province, Thailand

Methods

A community-based sample of Thai hill tribe women, aged 30-60 years, were recruited from 3 districts in Nan province, Thailand during the period of November, 9-12, 2015. A total of 712 women were enrolled to participate in the cervical cancer screening program using Surepath liquid-base cytology (BD, Becton and Dickinson) and Cobas 4800 HPV DNA testing (Roche, USA) followed by Linear array HPV genotyping test (Roche, USA) in HPV high risk (HR) non 16, 18 cases. Forty-four (6.2 %) women were excluded and 10 specimens (1.4 %) were inadequate for HPV result interpretation.

Results

Of the 658 eligible women, 5.0 % were positive for high risk HPV by Cobas 4800 HPV DNA testing. The overall prevalence of HR-HPV type 16, 18, non 16/18 was 1.1 %, 0.3 %, and 3.8 %, respectively. Using genotyping test, the most common detected

HR-HPV types excluding type 16, 18 were HPV-39 (0.9 %), HPV-66 (0.9 %), HPV-58 (0.8%), HPV-51 (0.5 %), and HPV-52 (0.5%). The abnormal PAP were found in 19 women (2.9%). The most common abnormal PAP included LSIL (1.4%), ASC-US (1.1%), ASC-H (0.3%) and HSIL (0.2%), respectively.

Conclusion

The most common HR-HPV genotype in this Thai hill tribe cohort was HPV-16, followed by HPV-39, HPV-66, HPV-58, HPV-51, HPV-52, and HPV-18. The prevalence of HR-HPV infection among hill tribe females in this study is quite low, compared with other studies in the western countries. Our results suggest using the HPV screening with triage by cytology in this population may lower unnecessary cervical cytology up to 95 %, leading to a potential benefit for supporting the use of HPV primary screening in Thailand.

OC 06-01

ANALYSIS OF IMMUNOGENICITY OF THE 9-VALENT HPV VACCINE BASED ON FIVE CLINICAL TRIALS

A. Luxembourg

Merck Sharp & Dohme, Corp. (United States of America)

Background / Objectives

Antibody response to the 9vHPV vaccine is the basis for its effectiveness in preventing infection and disease related to vaccine HPV types (HPV 6/11/16/18/31/33/45/52/58). Here we present the combined results of an integrated analysis of immunogenicity from several 9vHPV vaccine clinical trials.

Methods

Combined analyses of 5 clinical trials of the 9vHPV vaccine (protocols 001, 002, 005, 007 and 009) were conducted by competitive Luminex immunoassay (cLIA) in females aged 9-26 years and males aged 9-15 years in a per-protocol immunogenicity population (PPI) consisting of subjects seronegative at day 1 and (for those >15 years of age), PCR-negative from day 1 through 1 month post-dose 3 for the tested HPV type. Immunogenicity was summarized in populations defined by age at vaccination (≤ 15 or > 15 years of age) and gender.

Results

Of the randomized subjects, 11,304 (99.8%) received at least 1 injection. At least 99.6% of subjects who received 9vHPV vaccine and were in the PPI population were seropositive to the respective vaccine HPV type at 4 weeks post-dose 3. For all subjects, regardless of gender or age, geometric mean titers for all 9 HPV types were robust across race, ethnicity, or region. In general, the magnitude of anti-HPV response tended to decrease with an increase in enrollment age.

Conclusion

The 9vHPV vaccine was strongly immunogenic as shown by high seroconversion rates (>99%) for all vaccine HPV types and robust anti-HPV antibody responses regardless of race, geographic region, age or gender.

OC 06-02

INDUCTION OF IMMUNE MEMORY FOLLOWING ADMINISTRATION OF THE 9-VALENT HPV VACCINE

E. Joura

University of Vienna (Austria)

Background / Objectives

Duration of protection is an important parameter in the assessment of HPV vaccines. Vaccines that induce long term protection are generally characterized by the generation of immune memory. The pivotal efficacy study of the 9vHPV vaccine has shown high efficacy to prevent infection and disease due to the vaccine HPV types through at least 5 years. The purpose of this study is to assess the potential of the 9vHPV vaccine to induce HPV type specific immune memory.

Methods

This is an open-label extension of the pivotal efficacy study of 9vHPV vaccine (Protocol V503-001). A subset of subjects (N=150) initially randomized to 9vHPV vaccine and who received 3 dose regimen of 9vHPV vaccine in the base study were enrolled in the study extension and administered a 4th dose of 9vHPV vaccine at Month 60. HPV type specific serum antibody levels were measured pre-dose 4 and at 7 and 28 days post dose 4. Serum anti HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58 antibody levels were assessed by competitive Luminex immunoassay (cLIA).

Results

Pre-dose 4 geometric mean titers (GMTs) were lower than GMTs at Month 7 (4 weeks post dose 3) for all 9 vaccine HPV types. Administration of a fourth dose resulted in a rapid increase of GMTs for all 9 HPV types. GMTs at 7 and 28 days post dose 4 were higher than GMTs at 4 weeks post dose 3 for all 9 HPV types.

Conclusion

A three-dose regimen of the 9vHPV vaccine induces high efficacy and stable anti-HPV antibody levels for at least 5 years. Also, similar to what was previously observed with the quadrivalent HPV vaccine, administration of the 9vHPV vaccine induces robust immune memory. These findings suggest that the efficacy of the 9vHPV vaccine will be long lasting.

OC 06-03

COMPARISON OF IMMUNOGENICITY OF 2-DOSE AND 3-DOSE REGIMENS OF 9-VALENT HPV VACCINE

O.E. Iversen

Norwegian Inst of Tech Health Researc (Norway)

Background / Objectives

To compare HPV antibody responses in girls and boys aged 9-14 years who received 2 doses of 9-valent HPV (9vHPV) vaccine versus young women aged 16-26 years who received 3 doses.

Methods

Protocol V503-010 is an international, multi-centered, immunogenicity study of the 9vHPV vaccine. Subjects were enrolled in 5 cohorts: cohort 1: girls who received 2 doses of 9vHPV vaccine separated by a 6-month interval; cohort 2: boys who received 2 doses separated by a 6-month interval; cohort 3: girls and boys who received 2 doses separated by a 12-month interval; cohort 4 (control): young women who received 3 doses (at day 1, month 2, month 6); cohort 5 (exploratory): girls who received 3 doses (at day 1, month 2, month 6). HPV6/11/16/18/31/33/45/52/58 geometric mean titers (GMTs) and seroconversion rates were assessed at 1 month after the last dose using competitive Luminex immunoassay. The primary objectives of the study were to demonstrate non-inferior HPV antibody responses at 1 month after the last dose in cohort 1, cohort 2 and cohort 3 compared with cohort 4. The statistical criterion for non-inferiority required that the lower bound of the two-sided 95% confidence interval of GMT ratios (cohort 1/cohort 4, cohort 2/cohort 4, and cohort 3/cohort 4) be greater than 0.67 for each HPV type.

Results

Marked elevations of GMTs were elicited in all five cohorts at 1 month after the last dose. GMTs for all 9 HPV types were non-inferior in girls and boys aged 9-14 years who received 2 doses (cohorts 1, 2 and 3) compared with young women aged 16-26 years who received 3 doses (cohort 4). Therefore, the primary objectives of non-inferior immunogenicity of 2-dose vs. 3-dose regimens were met. Over 97.9% subjects seroconverted for all 9 vaccine HPV types. Exploratory analyses in girls aged 9-14 years showed that the 2-dose regimens (cohorts 1 and 3) resulted in lower GMTs than did the 3-dose regimen (cohort 5) for some of the HPV types.

Conclusion

Based on these non-inferiority results, and using the same approach as that previously accepted for licensure of 2-dose and 3-dose regimens of the quadrivalent HPV vaccine and the 3-dose regimen of the 9vHPV vaccine in girls and boys, efficacy findings in young women who received the 3-dose regimen of 9vHPV vaccine can be extended to girls and boys 9 to 14 years of age who received the 2-dose (0, 6) or (0, 12) regimen. No clinical significance can be assigned to the differences in GMTs between girls receiving 2 doses and girls receiving 3 doses since both groups had non-inferior immunogenicity compared with young women receiving 3 doses (the population and dose regimen used to establish 9vHPV vaccine efficacy).

OC 06-04

USE OF THE NONVALENT HPV VACCINE IN PREVIOUSLY VACCINATED INDIVIDUALS

P. Van Damme¹, P. Bonanni², X. Bosch³, E. Joura⁴, S. Krüger Kjaer⁵, C. Meijer⁶, K. Petry⁷, B. Soubeyrand⁸, T. Verstraeten⁹, M. Stanley¹⁰

¹Centre for Evaluation of Vaccination, Vaccine and Infectious Diseases Institute, University of Antwerp, Antwerp (Belgium), ²Department of Health Sciences, University of Florence, Florence (Italy), ³Cancer Research Epidemiology Program, Catalan Institute of Oncology, IDIBELL, Barcelona (Spain), ⁴Department of Obstetrics and Gynecology, Comprehensive Cancer Center Vienna, Medical University of Vienna, Vienna (Austria), ⁵Department of Gynaecology, Juliane Marie Centre, Rigshospitalet, Copenhagen University Hospital and the University of Copenhagen (Denmark), ⁶Department of Pathology, Free University Medical Center, Amsterdam (Netherlands), ⁷Department of Gynaecology and Obstetrics, Klinikum Wolfsburg, Wolfsburg (Germany), ⁸Sanofi Pasteur MSD, Department of Medical Affairs, Lyon (France), ⁹P95, Epidemiology and Pharmacovigilance Consulting and Services, Leuven (Belgium), ¹⁰Department of Pathology, Cambridge University, Cambridge (United Kingdom)

Background / Objectives

With the availability of the nonavalent human papillomavirus vaccine (9vHPV), parents, vaccinees, and vaccinators will be faced with questions on how to complete an immunization course started with the bi- or quadrivalent vaccine and whether to revaccinate individuals who have completed a full immunization course with the bi- or quadrivalent vaccine.

Methods

To address the questions raised on an individual level, four scenarios are proposed based on the age at the start of vaccination (9 to 14 years of age versus 15 years and older, the cut-off for 2 or 3 doses schedule), the number of doses already received and the time interval between those doses. Such individual guidelines are based on the European Summary of Product Characteristics (SmPC), available scientific data, and take into consideration guidelines made already by recommending bodies as well as expert opinions.

Results

As an example, the scenario for completing partially vaccinated girls, 9-14 years of age is shown (Table 1).

Table 1. Scenario sequential doses administration, for girls 9-14 years of age.

Month 0	Month 6	Month 12	Expected protection*
2vHPV			Incomplete
2vHPV	9vHPV		2 types
2vHPV	9vHPV	9vHPV	2 types and likely protection for the 7 extra types
4vHPV			Incomplete
4vHPV	9vHPV		4 types
4vHPV	9vHPV	9vHPV	4 types and likely protection for the 5 extra types

*: Expected according to currently available data and expert judgment; role of cross-protection is not taken into consideration

Although a single dose of vaccine may provide some protection[1], so far robust clinical data is lacking.

Conclusion

On an individual basis, the 9-valent vaccine can be used to complete an incomplete vaccination regimen or might be added to a previously completed schedule to extend protection. Finally, this position can also be applied to the vaccination of boys.

References

[1] Kreimer AR, Struyf F, Del Rosario-Raymundo MR, Hildesheim A, Skinner SR, Wacholder S, et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica Vaccine and PATRICIA trials. *Lancet Oncol.* 2015;16:775-86.

OC 06-05

EVALUATION OF THE INDIVIDUAL RESIDUAL RISK OF CERVICAL CANCER AFTER VACCINATION WITH GARDASIL 9.

K.U. Petry¹, **K. Bollaerts**², **P. Bonanni**³, **F.X. Bosch**⁴, **R. Drury**⁵, **E. Joura**⁶, **S. Kruger-Kjaer**⁷, **C. Meijer**⁸, **D. Riethmuller**⁹, **B. Soubeyrand**⁵, **P. Van Damme**¹⁰, **M. Stanley**¹¹

¹Department of Gynaecology and Obstetrics, Klinikum Wolfsburg, (Germany),
²P95, Epidemiology and Pharmacovigilance Consulting and Services (Belgium),
³Department of Health Sciences, University of Florence (Italy),
⁴Cancer Research Epidemiology Program, Catalan Institute of Oncology, IDIBELL (Spain),
⁵Sanofi Pasteur MSD, Department of Medical Affairs (France),
⁶Department of Obstetrics and Gynecology, Comprehensive Cancer Center Vienna, Medical University of Vienna (Austria),
⁷Department of Gynaecology, Juliane Marie Centre, Rigshospitalet, Copenhagen University Hospital and the University of Copenhagen (Denmark),
⁸Department of Pathology, Free University Medical Center (Netherlands),
⁹Pôle Mère-Femme, University Hospital Jean Minjot, Besançon (France),
¹⁰Centre for Evaluation of Vaccination, Vaccine and Infectious Diseases Institute, University of Antwerp (Belgium),
¹¹Department of Pathology, Cambridge University (United Kingdom)

Background / Objectives

Gardasil 9 is indicated for active immunisation of individuals from the age of 9 years against cervical, vulvar, vaginal and anal premalignant lesions and cancers causally related to vaccine HPV high risk (HR) types 16, 18, 31, 33, 45, 52 and 58. Gardasil 9 is anticipated to protect against the HPV types causing +- 90% of cervical cancers (CCs). The aim of this work was to estimate at individual level the residual country specific cumulative risk (CSCR) for developing CC and the risk of death from CC for an HPV naïve girl, unvaccinated or vaccinated with Gardasil 9 at the age of ten (i.e. considered HPV naïve), assuming lifelong vaccine protection and in absence of screening.

Methods

We used historical pre-screening age-specific CC incidence data from Denmark, Finland, Sweden and UK to calculate the CSCR of CC and mortality after vaccination with Gardasil 9 in the hypothetical absence of screening. To calculate the residual CSCR, the vaccine efficacy (VE) of 98.2% (95% CI: 93.5-99.8) for genotypes 16-18 and 97.1% (95%CI: 83.5-99.9) for the 5 additional HR types 31, 33, 45, 52 and 58 and genotype contribution fractions were used. To account for the uncertainty

in incidence rates, VE estimates and genotype contribution fractions, a Monte Carlo simulation model was built. Instead of using historical mortality rate, we calculated the mortality ratio using the most recent data on CC incidence and the related mortality rate.

Results

In absence of cervical screening, the CSCR of CC up to the age of 75 for unvaccinated girls varies from 1/31 [95% IC 1/29;1/34] (Denmark) to 1/76 [95% IC 1/73;1/78] (UK). For a girl vaccinated with Gardasil 9, the residual CSCR of developing CC is substantially reduced, with estimates from 1/238 [95% IC 1/171;1/314] (Denmark) to 1/572 [95% IC 1/413;1/748] (UK). Similarly, the CSCR of CC related mortality for unvaccinated girls varies from 1/117 [95% IC 1/93;1/149] (Denmark) to 1/205 [95% IC 189 ; 223] (UK); and reduces to values from 1/887 [95% IC 1/600;1/1268] (Denmark) to 1/1553 [95% IC 1/1114;1/2048] (UK) after vaccination.

Conclusion

Our simulation estimates that vaccination with Gardasil 9 before the age of HPV exposure reduces the individual life-time risk of cervical cancer and cervical cancer related mortality in European settings to very low levels. If applied to countries with cervical screening programs (notably HPV based) the reduction in risk would be greater, since HPV screening should identify precancerous lesions due to non-vaccine HPV types. In addition to the simulation modelled above, Gardasil 9 is anticipated to protect between 80% to 95% against HPV-related vulvar, vaginal and anal cancers, for which there is no screening program.

OC 06-06

**SUSTAINED NON-INFERIORITY OF IMMUNE
RESPONSE TO 2-DOSE SCHEDULES 0,6 AND 0,12
MONTHS (M) VERSUS 3 DOSES 0,1,6 M OF HPV-16/18
AS04-ADJUVANTED VACCINE; END OF STUDY
ANALYSIS OF A RANDOMIZED TRIAL**

L.M. Huang¹, T. Puthanakit², C.H. Chiu³, R.B. Tang⁴, T. Schwarz⁵, A. Pellegrino⁶, S. Esposito⁷, L. Frenette⁸, S. Mcneil⁹, P. Durando¹⁰, P. Rheault¹¹, C. Giaquinto¹², M. Horn¹³, K.U. Petry¹⁴, K. Peters¹⁵, A. Toma¹⁶, P. Hillemanns¹⁷, S. De Simoni¹⁸, D. Friel¹⁸, P. Suryakiran¹⁹, M. Hezareh²⁰, F. Thomas¹⁸, D. Descamps¹⁸, N. Folschweiller¹⁸, F. Struyf¹⁸

¹National Taiwan University Children's Hospital, National Taiwan University, Taipei (Taiwan, republic of china), ²Chulalongkorn University, Bangkok (Thailand), ³Chang Gung Children's Hospital, Chang Gung University, Taoyuan (Taiwan, republic of china), ⁴Cheng Hsin General Hospital, Taipei (Taiwan, republic of china), ⁵Stiftung Juliusspital, Würzburg (Germany), ⁶Azienda Sanitaria Locale Cuneo 1, Cuneo (Italy), ⁷Università degli Studi di Milano, IRCCS Fondazione Ospedale Maggiore Policlinico, Milan (Italy), ⁸Q&T Research Incorporated, Sherbrooke (Canada), ⁹Canadian Center for Vaccinology, IWK Health Centre and Nova Scotia Health Authority, Dalhousie University, Halifax (Canada), ¹⁰University of Genoa, Genoa (Italy), ¹¹Medicor Research Inc., Sudbury (Canada), ¹²University of Padova, Padova (Italy), ¹³Pediatric Office Dr. med. Michael Horn, Schoenau a. K. (Germany), ¹⁴Klinikum Wolfsburg, Wolfsburg (Germany), ¹⁵Facharzt für Frauenheilkunde und Geburtshilfe, Hamburg (Germany), ¹⁶Manna Research, Toronto (Canada), ¹⁷Medizinische Hochschule Hannover, Hannover (Germany), ¹⁸GSK Vaccines, Wavre (Belgium), ¹⁹GSK Pharmaceuticals India Ltd., Bangalore (India), ²⁰Chiltern International for GSK Vaccines, Wavre (Belgium)

Background / Objectives

This phase III, randomized, open-label, multi-centre trial (NCT01381575) previously demonstrated the non-inferior immunogenicity of HPV-16/18 AS04-adjuvanted vaccine when administered to girls as different 2-dose (2D) schedules versus 3-dose (3D) schedule in women, up to 24M post dose 1.¹ We report the immunogenicity and safety results 36M post dose 1.

Methods

Healthy girls (9–14 years [y]) were randomized 1:1 to receive 2D of HPV-16/18 AS04-adjuvanted vaccine at M0,6 or M0,12; a third group included healthy women (15–25y) who received 3D(M0,1,6). Anti-HPV-16/18 antibodies (by ELISA and pseudovirion-based neutralising assay [PBNA]) and T and B cell-mediated immune (CMI) responses were measured. Non-inferiority by ELISA was met if, for both HPV-16 and HPV-18, the upper limit of the 95% confidence interval for the seroconversion rate difference was <5% and for the geometric mean titre (GMT) ratio was <2.

Results

1285 subjects (506 in 2D[M0,6]; 378 in 2D[M0,12]; 401 in 3D[M0,1,6]) were included in the M36 according-to-protocol cohort for immunogenicity (ATP-I). At M36 in ATP-I initially seronegative subjects, 2D(M0,6) and 2D(M0,12) anti-HPV-16/18 responses were non-inferior to 3D(M0,1,6); 2D(M0,12) response was also non-inferior to 2D(M0,6) (Table). Anti-HPV-16/18 neutralising antibody levels at M36 appeared comparable or higher in 2D(M0,6) and 2D(M0,12) vs. 3D(M0,1,6). CMI responses at M36 were in the same range as 1M post vaccination in all groups (descriptive analyses). The vaccine safety profile remained clinically acceptable in all groups.

Table: Non-inferiority of the HPV-16/18 antibody responses (ELISA) in 2D(M0,6) and 2D(M0,12) vs 3D(M0,1,6) 36M after first vaccination		
	Anti-HPV-16 (95% CI)	Anti-HPV-18 (95% CI)
Seroconversion difference, % 3D(M0,1,6)–2D(M0,6)	0.00 (-1.15, 0.84)	-0.06 (-1.37, 0.96)
GMT ratio 3D(M0,1,6)/2D(M0,6)	1.10 (0.97, 1.24)	0.98 (0.85, 1.13)
Seroconversion difference, % 3D(M0,1,6)–2D(M0,12)	0.00 (-1.15, 1.12)	-0.28 (-1.58, 0.79)
GMT ratio 3D(M0,1,6)/2D(M0,12)	0.85 (0.74, 0.97)	0.69 (0.59, 0.80)
Seroconversion difference, % 2D(M0,6)–2D(0,12)	0.00 (-0.84, 1.12)	-0.22 (-1.22, 0.86)
GMT ratio 2D(M0,6)/2D(0,12)	0.78 (0.69, 0.87)	0.70 (0.62, 0.80)

Numbers in bold signify the non-inferiority criterion was met.

Conclusion

2D schedules of the HPV-16/18 AS04-adjuvanted vaccine administered in girls at 0,6M and 0,12M elicited a sustained and non-inferior immune response when compared to 3D schedule in young women, 36M after the 1st dose.

Funding: GlaxoSmithKline Biologicals SA

References

1. Tang RB et al. Abstract O9-011, APCMV 2015, Taipei, Taiwan.

OC 06-07

DESCRIPTION OF IGA/IGG IMMUNE RESPONSES DURING THREE DOSES OF THE HPV-16/18 AS04-ADJUVANTED VACCINE

A.K. Gonçalves¹, P.C. Giraldo², P.R. Machado¹, K.J. Farias¹, A.P. Costa¹, L.C. Souza¹, S.S. Witkin³

¹UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE-UFRN (Brazil),
²UNIVERSIDADE ESTADUAL DE CAMPINAS-UNICAMP (Brazil), ³WEILL CORNELL MEDICINE UNIVERSITY (United States of America)

Background / Objectives

This study was designed to describe the course of IgA/IgG responses in cervical secretions and in serum following intramuscular administration of the HPV-16/18 AS04-adjuvant vaccine.

Methods

The cervical samples for IgA and IgG antibody quantification were obtained by insertion and removal of 2 ml phosphate-buffered saline (PBS) in the cervical canal. Peripheral blood was collected by venipuncture. Blood was collected into tubes containing separating gel, coagulated at room temperature and then centrifuged at 3000xg to obtain serum. An ELISA for detection of IgA and IgG anti-HPV-VLP was developed for this purpose. The cut-off values were calculated using the median absorbance plus three times the standard deviation (SD) of the results obtained with negative serum or cervical mucus control obtained of healthy HPV negative women. Comparisons between median of absorbance for anti-VLP antibodies pre-vaccination and post-vaccination were performed using two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test. All values were considered significantly at $p < 0.05$. The software employed for analysis was GraphPad Prism software, version 6.0 (GraphPad Software Inc., EUA).

Results

IgG seroconversion after the second dose was observed in 100% of the subjects and remained one month after the third dose. Regarding IgG reactivity in cervical secretions, conversion was observed in 85% of women after the final dose. IgA seroconversion was observed in 76.7% of women after the third dose. Lower levels of IgA were detected in the cervical mucus (28.3%) and decreased to 23.3% after the last dose. Comparing local and systemic IgG responses, positivity in both serum and

cervical samples was observed in 85%, while in 15% only the serum was IgG antibody positive. A weak agreement between local and systemic IgA responses was observed. Only 18.3% of subjects were local and systemic IgA positive, 58.4% were positive only in serum, 5% were positive only in the cervix, and 18.3% were both local and systemic IgA antibody negative.

Conclusion

After the third vaccination, there is a strong agreement between cervical and systemic IgG antibody responses and a weak agreement between cervical and systemic IgA antibody responses. The induction of IgA antibodies appears to be secondary to that of IgG antibodies in response to HPV intramuscular vaccination. The similarity in antibody levels obtained after the second and third vaccination suggests that two vaccinations may be sufficient to protect against HPV infection.

OC 06-08

CROSS-PROTECTION & POPULATION EFFECT OF HPV VACCINES FROM THE PERSPECTIVE OF REAL-WORLD IMPACT DATA

G. Perez, A. Luxembourg, A. Saah

Merck & Co., Inc. (United States of America)

Background / Objectives

Real-World Impact (RWI) data provide evidence of a vaccine's ability to reduce infection and disease in a general population and help to inform policy and implementation decisions. Clinical trial data provided strong evidence regarding the efficacy of the quadrivalent and bivalent human papillomavirus (HPV) vaccines (4vHPV and 2vHPV vaccines). Some degree of cross-protection efficacy against non-vaccine HPV types was reported for both vaccines, although reported data showed a lack of consistent protection and short duration of that protection. In this review, we discuss cross-protection results in light of the RWI evidence.

Methods

Population-based studies and meta-analysis were reviewed to evaluate the impact of vaccination programs with high coverage and high series-completion on HPV prevalence, high-grade disease associated to non-vaccine types as well as high-grade lesions irrespective of HPV type.

Evidence from the meta-analysis with more than 140 million person-years of follow-up data supports the role of HPV vaccination in decreasing Genital Warts Incidence and HPV 16/18 prevalence which fell by 72% (RR: 0.28 (0.14–0.56)) in the post-vaccination period, compared to the pre-vaccination period, in women 13 to 19 years of age receiving 4vHPV.

Results

Partial though significant reductions in HPV types 31 and 45 were reported after 4vHPV in Australia (P=0.0029 and P=0.0043 for HPV 31 and 45, respectively); similar results were reported for 2vHPV showed statistically significant reductions in the prevalence of HPV 31, 33 or 45 in women fully vaccinated in Scotland (P=0.0002; P=0.002; P=0.001, respectively) and non-significant results in the UK.

Reductions of CIN1 by 29%, CIN2 by 50%, and CIN3 by 55% in 20-21 year-old women vaccinated with 2vHPV in Scotland and up to 80% reduction in CIN 2/3 and CIN 3 in women aged before 20 years in Denmark with 4vHPV showed the overall effect on high-grade disease irrespective of HPV type.

Conclusion

RWI data support high efficacy against VLP-based vaccine HPV types and partial cross-protection effect against few non-vaccine HPV types for both vaccines. Overall reduction in CIN2/3 and CIN3 due to HPV types included as well as those not included in the vaccines suggest that even in short-term RWI data, the effect of cross-protection is modest. Furthermore, the high efficacy results attributed to cross-protection and referred to as Population Impact in a single clinical trial are not observed in RWI studies. The nine-valent HPV vaccine directly targets the most common oncogenic HPV types, making short-duration cross-protection even less relevant for public health programs.

OC 06-09

60 MONTH FOLLOW UP OF A TWO DOSE HPV-4 VACCINE SCHEDULE; RESULTS FROM A PHASE III POST-LICENSURE RANDOMIZED TRIAL

S. Dobson¹, G. Ogilvie¹, C. Sauvageau², M. Dionne², V. Gilca², S. Mcneil³, M. Kraiden¹, D. Money¹

¹University of British Columbia, Vancouver, British Columbia (Canada), ²Centre Hospitalier Universitaire de Québec-Université Laval, Quebec City, Quebec (Canada), ³Dalhousie University, Halifax, Nova Scotia (Canada)

Background / Objectives

In a phase III randomized trial, 2 doses of quadrivalent HPV (HPV-4) given to 9-13 year old girls (0,6months (M)) was non-inferior to 3 doses given to 16-26 year old women (0,2,6M). In the original trial, we also included a 3 dose arm for girls 9-13 year old (0,2,6M). In this study, we report the 60M follow up after first dose for the 2- and 3-dose girls.

Methods

Participants provided a blood sample at 60M. Sera were evaluated using Merck Competitive Luminex ImmunoAssay (cLIA) and total IgG LIA (Luminex Immunoassay) assays to assess serum antibodies to HPV-6,11,16 and 18. Seropositivity (SP) rates and geometric mean titre (GMT) for genotypes were compared between 2- and 3-dose recipients. The 2-sample t-test with unequal variances was used to compute p-values.

Results

101 girls provided blood samples (50:2 dose;51:3 dose group). With the exception of HPV-18, seropositivity was >95% for other genotypes. There was no significant difference in seropositivity between 2 and 3 dose girls. There was no significant difference in GMTs using IgG or cLIA between 2- and 3-dose recipients with the exception of HPV-18 cLIA (p=0.04).

Type	Assay	2 Dose SP % (95% CI)	3 Dose SP % (95% CI)	2 Dose GMT* (95% CI)	3 Dose GMT*(95% CI)
6	cLIA	96 (86-100)	98 (90-100)	150 (114-198)	205 (157-269)
6	IgG	100 (93-100)	100 (93-100)	207 (147-291)	242 (180-327)
11	cLIA	100 (93-100)	100 (93-100)	223 (167-298)	225 (174-289)

11	IgG	98 (89-100)	98 (90-100)	163 (120-220)	170 (125-230)
16	cLIA	100(93-100)	100 (93-100)	949 (691-1304)	829 (628-1094)
16	IgG	100 (93-100)	100 (93-100)	904 (668-1223)	735 (541-998)
18	cLIA	84 (71-93)	94 (84-99)	78 (53-117)	137 (94-198)
18	IgG	86 (73-94)	92 (81-98)	107 (73-158)	143 (99-204)

*GMT for cLIA and LIA is expressed as milli Merck Units/ml

Conclusion

At 60M post dose 1, there was no significant difference in SP or GMTs between 2- and 3-doses girls ages 9-13 year, with the exception of HPV-18 cLIA GMTs. HPV-18 IgG GMTs however, were not significantly different. 2- and 3-dose SP remains relatively unchanged from the 36M rates in these groups (data not shown). This analysis provides reassurance for programs using 2 doses HPV-4 that immunogenicity is comparable to 3-doses at 60M after dose 1. These study participants are also a subgroup of a larger cohort under evaluation for effectiveness of the 2-dose HPV-4 vaccine, which will provide further guidance regarding the impact of reduced dose schedules.

OC 06-10

SUSTAINED ANTIBODY RESPONSES SIX YEARS FOLLOWING REDUCED DOSE QUADRIVALENT HPV VACCINE IN ADOLESCENT FIJIAN GIRLS

Z.Q. Toh¹, F.M. Russell², R. Reyburn¹, J. Fong³, E. Tuivaga³, T. Ratu³, C.D. Nguyen¹, R. Devi³, M. Kama³, M. Silivia³, S.N. Tabrizi⁴, S.M. Garland⁴, R. Sinha⁵, I. Frazer⁵, L. Tikoduadua³, J. Kado³, E. Rafai³, E.K. Mulholland⁶, P.V. Licciardi¹

¹Murdoch Childrens Research Institute (Australia), ²Murdoch Childrens Research Institute;The University of Melbourne, Department of Paediatrics, Centre for International Child Health (Australia), ³Ministry of Health and Medical Services (Fiji), ⁴The Royal Women's Hospital and Murdoch Childrens Research Institute, Department of Obstetrics and Gynecology; The Royal Women's Hospital Regional, WHO HPV Reference Laboratory, Department of Microbiology and Infectious Disease (Australia), ⁵The University of Queensland Diamantina Institute (Australia), ⁶Murdoch Childrens Research Institute;London School of Hygiene and Tropical Medicine;Menziess School of Health Research, Department of Child Health (Australia)

Background / Objectives

Recently, the World Health Organization has recommended two dose HPV vaccine schedule separated by 6 months to girls <15 years old as an alternative to the current three dose schedules. However, the long-term protection following reduced dose schedules is unknown. This study examined long-term immunity by comparing the antibody responses in girls previously given 3 doses of 4vHPV (Gardasil®, Merck Inc.) 6-7 years ago with reduced doses (1 or 2 doses).

Methods

A prospective cohort study was undertaken in 200 Fijian girls (15-19 years old) who previously received 0, 1, 2 or 3 doses of 4vHPV 6-7 years ago (N=50/group). Blood was taken pre- and 28 days following a single dose of 2vHPV (Cervarix®, GSK), and HPV 6, 11, 16 and 18 neutralising antibodies (NAb) were measured using the pseudovirion neutralisation assay.

Results

Geometric Mean NAb Titres (GMT) were similar for HPV 16 and 18 between the 2 and 3 dose recipients: HPV 16 (3 doses: 3575; 2 doses: 2904; p=0.37); HPV 18 (3

doses: 687; 2 doses: 543; $p=0.41$). Single 4vHPV dose recipients had higher HPV 16 and 18 NAb titres than unvaccinated girls (HPV 16 and 18: $p<0.001$). Post- 2vHPV NAb titres against HPV 16 and 18 increased at least 14- and 39-fold, respectively to a level that were similar between groups previously given 1, 2 or 3 doses of 4vHPV (HPV16 GMT: 49,114-51,915; HPV 18 GMT: 22,286-29,076). Interim analysis for HPV6 and 11 showed a similar dosage effect prior to 2vHPV between the groups after 6-7 years, but with no increase in NAb titres following 2vHPV.

Conclusion

A dose-response in NAb titres was observed in girls given 1, 2 or 3 doses of 4vHPV 6-7 years previously, but there was no significant difference in NAb titres between the 2 and 3 dose groups. A single 4vHPV dose produced substantial HPV16 and 18 NAb titres that were 6- and 3-fold, respectively, higher than unvaccinated individuals after 6-7 years, suggesting a possible benefit of a single dose, although the clinical significance is unknown. Interim analysis showed no evidence of cross-neutralising antibodies against HPV 6 and 11 in all groups following a single dose of 2vHPV. Assays are being developed to determine cellular responses. This information supports the current two dose 4vHPV schedule.

OC 06-11

COST-EFFECTIVENESS EVALUATION OF THE QUADRIVALENT HPV VACCINATION PROGRAM FOR FEMALES AGE 9-10 YEARS IN COSTA RICA

D. Medina¹, **A.S. Kulkarni**², **A. Pavelyev**³, **A. Rosado Buzzo**⁴, **G. Luna Casas**⁴, **R. Navarro Rodriguez**⁴, **M. García Mollinedo**⁴, **M. Lutz**⁵, **H. Monsanto**⁶, **D. Guarin**⁷, **M. Cashat**⁸, **M.A. O'brien**², **M. Pillsbury**⁹

¹Hospital Calderón Guardia, Costa Rica (Costa rica), ²Center for Observational and Real World Evidence, Merck, USA (United States of America), ³HCL America Inc. (United States of America), ⁴Links & Links (Mexico), ⁵MSD Costa Rica (Costa rica), ⁶MSD Puerto Rico (Puerto rico), ⁷MSD Colombia (Colombia), ⁸MSD Mexico (Mexico), ⁹Health Economics and Statistics, Merck, USA (United States of America)

Background / Objectives

To examine cost effectiveness of quadrivalent human papillomavirus (HPV) vaccine compared with bivalent vaccine and no vaccination (screening only) in Costa Rica.

Methods

Previously published dynamic transmission model was adapted and calibrated for Costa Rica. The HPV model simulated the natural history of cervical cancer and genital warts in Costa Rica. It was assumed that the vaccination program would be combined with current cervical cancer screening in Costa Rica. For the model, it was assumed that 70% of girls 9-10 years would receive two doses of HPV vaccine. The relative effectiveness of two doses of vaccine was assumed to be the same as three doses. Impact of HPV types 6/11/16/18 only were considered for this model. The quadrivalent HPV vaccination program (combined with cervical cancer screening) was compared with a program for cervical cancer screening only (no HPV vaccination) and with a bivalent vaccination program (combined with cervical cancer screening). Life-long duration of protection was assumed for both HPV vaccines.

Results

The quadrivalent HPV vaccines resulted in the reduction of HPV types 6/11 related genital warts in females (77%) and males (75%) and CIN1 (76%). The implementation of the quadrivalent vaccination program was cost saving as compared to the bivalent vaccination program and the program for cervical cancer

screening only. The quadrivalent vaccination program strongly dominated the bivalent vaccination program and the program with cervical cancer screening only.

Conclusion

In Costa Rica, vaccinating 9-10 year old girls with the quadrivalent HPV vaccine has additional public health impact and is cost saving as compared with the bivalent HPV Vaccine program or the screening only program.

OC 07-01

THE STATE OF THE ART OF HPV ASSOCIATION IN NON-ORO-GENITAL CANCERS. AN OVERVIEW.

K. Syrjänen

Department of Clinical Research, Biohit HealthCare Oyj; Helsinki, Finland (Finland)

Background / Objectives

The first reports suggesting HPV involvement in development of sino-nasal (SNC), laryngeal (LC), bronchial (BC) and esophageal cancer (EC) date back to the late 1970's and early 1980's. This overview summarizes the evidence accumulated during the past 35 years, based on timely meta-analytical data of the published literature.

Methods

Literature published on HPV detection in sino-nasal-, laryngeal-, bronchial- and esophageal cancers and their benign counterparts (papillomas) was subjected to 7 separate meta-analyses published in 2012-2015. In all reports, the effect size was calculated as event rates (95% CI), with homogeneity testing using Cochran Q and I² statistics. Meta-regression was used to test the impact of study-level covariates.

Results

Of sino-nasal carcinomas, 35 eligible studies, covering 492 SCCs from different geographic regions were included. Of those, 133 (27.0%) cases tested HPV-positive; effect size 0.305 (95% CI, 0.260-0.355; FE model), and 0.330 (95% CI, 0.249-0.423; RE model). Of laryngeal SCCs, 180 studies were eligible comprising a sample size of 7,353 larynx squamous cell carcinomas from different geographic regions. Of these cases, 1,833 (25%) cases tested HPV-positive considering all methods; effect size 0.269 (95%CI 0.242-0.297; RE model). Exactly 100 studies on lung cancer were eligible, covering 7,381 lung cancer cases (different histological types). In total, 1,653 (22.4%) samples tested HPV-positive; effect size was 0.348 (95% CI=0.333-0.363; FE model), and 0.220 (95% CI=0.18-0.259; RE model). Of bronchial papillomas, 15 studies were eligible, covering 89 bronchial SCPs. Altogether, 38 (42.7%) cases tested HPV-positive; effect size 0.422 (95% CI: 0.311–0.542; FE model), and 0.495 (95% CI:0.316–0.675; RE model). Of the 1,177 abstracts found on ESCC, 152 studies were determined to be eligible. These 152 studies covered a total of 10,234 ESCC cases, analysed by different HPV detection methods in different geographic regions. Altogether, 3,135 (30.6%) tested HPV-positive, translating to an effect size

of 0.372 (95% CI 0.360 – 0.384; FE model) and 0.290 (95% CI 0.251 – 0.31; RE model). Of ESCPs, 39 studies were eligible, covering 427 ESCPs from different geographic regions. In total, 132 (30.9%) cases tested HPV positive; effect size 0.375 (95% CI 0.319–0.434) using FE model and 0.412 (95% CI 0.295–0.540) using RE model

Conclusion

The results of the 7 published meta-analysis indicate that the reported wide variability in HPV detection in NSC, LC, BC and EC is not due to the HPV detection techniques, but is best explained by the geographic origin of the study, except in SNC. In LC, also the histological type is a significant study-level covariate.

OC 07-02

GENOMIC ANALYSIS OF HPV-POSITIVE VERSUS HPV-NEGATIVE OESOPHAGEAL ADENOCARCINOMA IDENTIFIES A DIFFERENTIAL MUTATIONAL LANDSCAPE

S. Rajendra¹, **B. Wang**¹, **N. Merrett**², **P. Sharma**³, **J. Humphris**⁴, **H.C. Lee**⁴, **J. Wu**⁴

¹Ingham Institute for Applied Medical Research, University of New South Wales (Australia), ²Western Sydney University (Australia), ³University of Kansas City (United States of America), ⁴Garvan Institute of Medical Research, University of New South Wales (Australia)

Background / Objectives

High-risk human papillomavirus (hr-HPV) has been implicated in a subset of patients with oesophageal adenocarcinoma (OAC).¹⁻³ We therefore hypothesized that HPV associated OAC may have a distinct distribution of molecular aberrations and genomic abnormalities compared with HPV negative oesophageal cancer. As such, whole exome sequencing (WES) was performed to explore the genomic landscape and potential molecular signature of HPV positive versus viral negative OAC.

Methods

Four hr-HPV-positive and 8 HPV-negative treatment-naïve fresh frozen OAC tissue specimens and matched normal tissue were analysed by WES to identify somatic genomic mutations. Data were subjected to cancer driver gene identification, pathway analysis and detection of virus genome integration.

Results

The HPV-positive cohort harboured approximately 50% less non-silent somatic mutations than the virus-negative oesophageal cancer patients (1.31 mutations/Mb vs 2.56 mutations/Mb, $p=0.048$). TP53 aberrations were absent in the HPV-positive OAC group whereas 50% of the HPV-negative OAC patients exhibited TP53 mutations. Viral integration analysis identified hybrid sequences containing HPV16 and the human genome. HPV-negative cancers were enriched with non-silent mutations in cancer driver genes, but not HPV-positive tumours. Enriched A>C transversions at AA dinucleotide was observed in 5/7 Siewert class I OAC samples but none (0/5) in Siewert class II tumours ($p=0.027$).

Conclusion

These findings demonstrate distinct genomic differences between HPV-positive and HPV-negative OAC indicating different biological mechanisms of tumour formation.

References

1. Rajendra, S. et al. Transcriptionally active human papillomavirus is strongly associated with Barrett's dysplasia and esophageal adenocarcinoma. *Am J Gastroenterol* 2013; 108: 1082-93.
2. Wang, B. et al. Viral load and integration status of high-risk human papillomaviruses in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. *Am J Gastroenterol* 2013; 108: 1814-6.
3. Rajendra, S. et al. Persistence of Human Papillomavirus, Overexpression of p53, and Outcomes of Patients After Endoscopic Ablation of Barrett's Esophagus. *Clin Gastroenterol Hepatol* 2015; 13: 1364-1368.e5

OC 07-03

PERSISTENT HUMAN PAPILLOMAVIRUS DETECTED IN BREAST MILK

K. Louvanto¹, M. Sarkola¹, M. Rintala², K. Syrjänen³, S. Grenman², S. Syrjänen⁴

¹Helsinki University Hospital, Department of Obstetrics and Gynecology, Helsinki (Finland), ²Turku University Hospital, Department of Obstetrics and Gynecology, Turku (Finland), ³Biohit Oyj, Department of Clinical Research, Helsinki (Finland), ⁴University of Turku, Department of Oral Pathology and Radiology, Turku (Finland)

Background / Objectives

HPV DNA has been detected in breast milk but its origin has remained obscure. The aim of the study was to analyze the prevalence and persistence of HPV in breast milk during the first 6 month follow-up (FU) of the Finnish Family HPV cohort study. The association of breast milk HPV persistence with women's oral and genital HPV status as well as with oral HPV status of her infant was further evaluated.

Methods

We included 308 mothers and their newborns to the study. Mothers collected the milk samples manually at day 3, month 2 and 6. Cervical and oral samples were taken before delivery, and at month 2 and 6. Oral samples from newborns were taken at delivery, day 3 and, at month 2 and 6. HPV testing was done with nested PCR and Luminex-based Multimetrix kit.

Results

Breast milk HPV DNA was found in 10.1% (31/308), 20.1% (39/194) and 28.8% (17/59) at day 3, month 2 and 6, respectively. Of the HPV genotypes, HPV16 and HPV6 was found at day 3, HPV16 and HPV33 at month 6, while at month 2 HPV 16,18,45,53,56,59,66 and 82 were detected. Breast milk HPV persisted among 5.5% (9/164) of the women. 77.8% (7/9) of these breast milk HPV persistent infections were a single or co-infection with HPV16. At baseline, these breast milk persistors were HPV6 seropositive and HPV16 seronegative except one. Their oral samples were always HPV-negative as were cervical samples except one. 55.6% (5/9) of the breast milk persistors' children got an incident oral HPV infection during the FU. Among these children two had a persistent oral infection with HPV16. Due to the small number of breast milk persistors no significant associations were detected

between the persistent breast milk and the children's oral incident or persistent HPV infection.

Conclusion

To conclude, HPV16 can persist in breast milk independent of the mother's oral HPV status. The breast milk might have an effect on the breast milked children's future oral HPV status.

OC 07-04

HUMAN PAPILOMAVIRUS 16 IS AN ETIOLOGICAL FACTOR OF SCROTAL CANCER.

N. Guimera¹, **L. Alemany**², **G. Halec**³, **M. Pawlita**⁴, **G.V. Wain**⁵, **J. Santos Salas Vailén**⁶, **J. Azike**⁷, **D. Jenkins**¹, **S. De Sanjosé**², **W. Quint**¹, **F.X. Bosch**⁸

¹DDL Diagnostic Laboratory, Rijswijk. (Netherlands), ²IDIBELL, Institut Català d'Oncologia - Catalan Institute of Oncology, L'Hospitalet de Llobregat (Barcelona), Spain./ CIBER en Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain. (Spain), ³Division of Genome Modifications and Carcinogenesis, Infections and Cancer Program, German Cancer Research Center, Heidelberg, Germany / ⁵. Department of OB/Gyn, UCLA AIDS Institute, Los Angeles, CA, USA. (Germany), ⁴Division of Genome Modifications and Carcinogenesis, Infections and Cancer Program, German Cancer Research Center, Heidelberg, Germany. (Germany), ⁵Gynaecological Oncology, Westmead Hospital, Westmead, New South Wales, Australia. (Australia), ⁶Hospital de León, León, Spain. (Spain), ⁷College of Medicine, Imo State University Teaching Hospital, Orlu Campus, Nigeria / Department of Surgery, Imo State University Teaching Hospital, Orlu, Nigeria. (Nigeria), ⁸IDIBELL, Institut Català d'Oncologia - Catalan Institute of Oncology, L'Hospitalet de Llobregat (Barcelona), Spain. (Spain)

Background / Objectives

Scrotal carcinoma is a very rare skin cancer. This carcinoma has historically been associated with exposure to environmental or industrial carcinogens and has only rarely been associated with human papillomavirus (HPV). To determine the etiological relation between HPV and scrotal cancer we performed a comprehensive evaluation of the HPV involvement by using different genotyping techniques at the DNA and RNA level, and accurately establishing the location of HPV in the tumor cells. P16^{INK4a} overexpression was studied to determine its relation with HPV positive neoplasias and p53 expression to determine if this biomarker was mutated in HPV negative neoplasias.

Methods

Six scrotal carcinomas have been collected from Spain (n=2), Australia (n=2) and Nigeria (n=2) with a median patient age of 60.5 years. FFPE specimens were sectioned by sandwich technique under strictly non-contamination conditions for histological diagnosis, whole tissue PCR analysis and laser capture microscopy-PCR (LCM-PCR). Sections were stained by haematoxylin and eosin, for p16^{INK4a} and for

p53. Independent pathologists evaluated histopathology and immunohistochemistry. HPV analyses on whole tissue sections was performed at the DNA level by SPF10-DEIA-LiPA25 (version 1), by MPTS 123 PCR-Luminex assay, by Beta HPV assay and Cutaneous Wart-Associated HPV; and at the RNA level by E6*1 mRNA Reverse Transcription (RT)-PCR and luminex genotyping system. To evaluate DNA/RNA quality and PCR inhibition RNaseP/PhHV qPCR and transcript ubiquitin C system.

Results

Squamous cell carcinoma was the only histological type identified. HPV genotyping analyses (DNA/RNA) on whole tissue sections reveal the single presence of HPV16 in three out of six cases. Subsequently, LCM-PCR analysis on these three positive cases confirmed the presence of HPV16 in tumor cells. P16^{INK4a} was overexpressed on these three HPV16 positive cases and p53 was overexpressed (75%) in two out of three HPV negative tumors. From one of these three HPV positive patients metastatic biopsies were available and in all of them HPV16 presence was confirmed with the same methodology

Conclusion

HPV16 is mostly associated to mucosal malignant lesions and it has only very occasionally been associated with scrotal cancer. This is the first time that HPV16 has been shown to be definitively present in invasive neoplastic cells of cutaneous cancer from the scrotum. A decrease in scrotal cancer incidence was expected with improved occupational hygiene and the removal of chemical carcinogens. However, such tumors still occur infrequently due to HPV infection.

OC 07-05

HUMAN PAPILOMAVIRUS SYNERGISTIC ASSOCIATION WITH KRAS TOWARDS PROMOTING ABERRANT DNA METHYLATION IN COLORECTAL ADENOCARCINOMA

S. Ghosh

Department of Biotechnology, Assam University, Silchar (India)

Background / Objectives

Colorectal Cancer (CRC) is the fourth leading cancer world-wide with approximately 1,360,602 new cases annually. The earliest known model of sporadic CRC includes the adenoma-carcinoma sequence characterized by chromosomal instability and alterations in specific oncogenes (e.g. KRAS) or tumour suppressor genes (e.g. TP53, APC etc). More recently, alternative molecular pathways characterised by multiple CpG Island Methylation (termed as CIMP) associated with distinct genetic alterations in BRAF and KRAS oncogenes are also identified. The interaction of HPV with frequently perturbed molecular events in colorectal cancer development has not been investigated in details.

Methods

The present studied the association of HPV with various genetic (MSI, KRAS, TP53 and BRAF mutations) and epigenetic (CpG island methylation) events colorectal cancer. HPV DNA was detected by PCR using My09/My11 and Gp5+/Gp6+ consensus primers and typed using HPV16 and HPV18 specific primers. MSI was detected using BAT 25 and BAT 26 markers and mutation of KRAS, P53 and BRAF V600E was detected by direct sequencing. Methyl specific polymerase chain reaction was used to determine promoter methylation of the classical CIMP panel markers (P16, hMLH1, MINT1, MINT2 and MINT31) and other tumour related genes (DAPK, RASSF1, BRCA1 and GSTP1).

Results

HPV DNA was detected in 34/93 (36.5%) colorectal tumour tissues, HPV 18 being the predominant high-risk type. HPV presence was not associated with age, stage or grade of tumours, MSI or mutations in KRAS, P53 or BRAF genes; however a non-significant trend for better survival was observed in HPV positive cases. HPV was not only found to be associated with CIMP-high in both univariate and multivariate analysis, but also a synergistic association was observed between HPV and KRAS

codon12, 13 mutations (OR= 8.01 [2.05-31.27], P=0.003) towards promoting aberrant CpG island methylation in colorectal cancer.

Conclusion

The present findings indicate that, KRAS oncogenic mutations might synergistically co-operate HPV to promote CpG island methylation in high-risk HPV associated colorectal cancer progression. HPV therefore, might be a potent risk factor of colorectal malignancies in developing countries and epigenetic deregulation of host genome might be an alternate pathway for HPV pathogenesis in CRC.

OC 07-06

HPV-RELATED GENITAL DISEASE AMONG MEN RESIDING IN BRAZIL

R.J. Carvalho Da Silva ¹, S.L. Sudenga ², M.L. Baggio ³, L. Galan ³, R. Cintra ³, L. Sicheo ³, E. Lazcano-Ponce ⁴, A.R. Giuliano ², L.L. Villa ⁵

¹Centro de Referência e Treinamento em DST/Aids- São Paulo (Brazil), ²Moffitt Cancer Center & Research Institute, Tampa, FL, USA (United States of America), ³Center for Translational Research in Oncology, Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil (Brazil), ⁴Instituto Nacional de Salud Pública, Cuernavaca, Mexico (Mexico), ⁵School of Medicine, Universidade de São Paulo, São Paulo, Brazil (Brazil)

Background / Objectives

The purpose of this study was to assess HPV infection associated with the development of external genital lesions (EGL), specifically, condyloma (genital warts) and penile intraepithelial neoplasia (PeIN), in healthy men aged 18–70 residing in São Paulo (Brazil), within the multinational HIM Study. We assessed the incidence of pathologically confirmed condyloma and PeIN and determined the rate of HPV infection progression to EGL. Factors associated with each of these outcomes were assessed.

Methods

The Brazil HIM Study participants are men aged 18-70 years living in São Paulo (Brazil) enrolled between July 2005 and June 2009. At each visit, visually distinct EGLs were biopsied, subjected to pathological evaluation, and categorized by pathological diagnoses. Genital swabs and biopsies were used to identify HPV types using PCR and the Linear Array genotyping method for swabs and INNO LiPA for biopsies. Condyloma and PeIN incidence was determined among 1,118 men aged 18-73 years with two or more visits.

Results

Among the Brazil cohort, 73 men developed an incident EGL during the study period. Men could develop multiple types of EGLs primarily diagnosed as condyloma (n=36), PeIN (n=6), and non-HPV related EGL (n=20). Men that developed an EGL were younger in age ($p=0.006$) and were single ($p=0.01$) compared to men that did not develop an EGLs.

During follow-up of 815 men with an HPV infection, 4% progressed to an EGL with the same HPV type detected in the lesion. Genital HPV-6 progression to an HPV-6-positive condyloma was 15.7% (18/115) with a median time from infection to condyloma of 9.0 months. Similar results were obtained for HPV-11 infections. Less than 1% (0.2%, 4 of 2232) of any genital HPV infections progressed to PeIN with the same HPV type detected in the lesion, with a median time from infection to PeIN of 24.9 months. Approximately 1% of genital HPV-16 infections progressed to an HPV-16-positive PeIN with a median time from infection to PeIN of 25.6 months. The quadrivalent HPV vaccine types were detected in 82.3% and 83.3% of condyloma and PeIN respectively.

Conclusion

EGL incidence in Brazilian men was associated with young age and being single. Most EGLs develop following infection with HPV types 6, 11, or 16, one to two years from HPV detection. This study emphasizes the need to ensure that strong HPV immunity, such as that obtained through vaccination, is available to males to reduce the overall burden of infection and diseases caused by HPV.

OC 07-07

The Prognostic Impact of HPV Infection on Vulvar Cancer outcome

K. Wakeham¹, K. Cuschieri², S. Graham¹, K. Kavanagh³, D. Millan⁴, S. Bell⁴, K. Pollock⁵

¹University of Glasgow (United kingdom), ²Scottish HPV Reference Laboratory, NHS Lothian (United kingdom), ³University of Strathclyde (United kingdom), ⁴Greater Glasgow and Clyde NHS (United kingdom), ⁵Health Protection Scotland (United kingdom)

Background / Objectives

It is universally accepted that high-risk human papillomavirus (HR-HPV) is the cause of cervical dysplasia and cancer. More recently it has been shown that HPV is also a marker of clinical outcome in oropharyngeal cancer. However, contemporary information is lacking on both the prevalence of HPV infection in vulvar cancer (VSCC) and the influence of HPV-status on the prognosis of this malignancy.

Methods

We retrieved tissue from all cases of VSCC diagnosed between 01/01/2008 and 31/12/2009 from a regional (NHS Greater Glasgow and Clyde) pathology biobank. All tissue underwent pathology review and clinical variables were extracted from electronic records. HPV genotyping using the Optiplex HPV Genotyping assay was performed. This assay detects 24 HPV types including all established high-risk types. Rates of overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan–Meier method. Cox proportional-hazards models were used to estimate hazard ratios.

Results

Valid HPV results were obtained for 62/66 cases of VSCC; HPV infection was present in 52% cases (32/62) and all types identified were HR-HPV types. As age increased, VSCC cases were less likely to be associated with HR-HPV (OR 0.51 95% CI 0.33-0.80, p trend=0.003). The mean follow-up time was 6 years. In the Kaplan-Meier analysis, women with HR-HPV positive VSCC had better OS (log-rank test p=0.01) and PFS (log-rank test p=0.0001). The 5-year rates of OS were 78% in the HR-HPV positive group and 49% in the HPV negative group. The 5-year rates of PFS were 87% in the HR-HPV positive group and 47% in the negative group. After adjustment for age and cancer stage, patients with HPV positive tumours had a 67%

reduction in risk of death (hazard ratio, 0.33; 95% CI 0.12-0.80, $p=0.001$) and an 83% reduction in risk of disease progression (hazard ratio, 0.18; 95% CI 0.07-0.48, $p=0.01$) compared to HPV negative tumours.

Conclusion

HPV infection is common in vulvar cancer, especially in the young. HPV positivity is strongly associated with a better prognosis in patients with vulvar cancer even after adjustment for age and cancer stage.

OC 07-08

Patterns of distant metastases in vulvar cancer

K. Prieske¹, **N. Haeringer**¹, **D. Grimm**¹, **F. Trillsch**¹, **C. Eulenburg**², **E. Burandt**³, **B. Schmalfeldt**¹, **S. Mahner**⁴, **V. Mueller**¹, **L. Woelber**¹

¹Department of Gynecology and Gynecologic Oncology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg (Germany),

²Department of Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg (Germany), ³Department of Pathology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg (Germany), ⁴Department of Gynecology and Obstetrics, University of Munich, Marchioninstr. 15 81377 München (Germany)

Background / Objectives

While treatment of early stage vulvar cancer is standardized, little information is available regarding treatment of advanced, recurrent and especially metastatic disease. We therefore analyzed incidence and pattern of metastases in vulvar cancer in a large retrospective single center cohort.

Methods

All patients with primary squamous cell cancer of the vulvar (n=391, median age: 60 years; range 20-94) treated at the Gynecologic Cancer Center Hamburg-Eppendorf between 1996 and 2013 were retrospectively evaluated for occurrence of distant metastasis. Pattern of metastatic spread, prognosis and therapeutic strategies were analyzed.

Results

Out of 391 patients with primary squamous cell vulvar cancer, 20 patients (5.1%) developed distant metastases. Median time to diagnosis of metastases was 13.5 months (range 4-104) after primary diagnosis. Most patients experienced one or more local recurrences before distant spread (12/20, 60%). Documented metastatic sites were lung (n=9), liver (n=7), bone (n=5), lymph-nodes (axillary, thoracic, paraaortic, n=3) and skin (n=4). The majority of patients presented with unilocal metastases (n=13). In univariate analysis tumor diameter, invasion depth, nodal status and number of metastatic lymph nodes were identified as predictive factors for the occurrence of distant metastases. Two-year overall survival rate of all metastatic patients was 12.5%, median survival from first diagnosis of metastases 5.6 months.

Conclusion

The occurrence of distant metastases from vulvar cancer is a rare event with limited prognosis. Further research efforts will be crucial to identify prognostic markers as well as therapeutic targets in order to improve survival in these patients.

OC 07-09

Vulvar Cancer: HPV DNA and survival

L. Martins¹, D. Cochicho¹, A. Sofia², A. Coelho², M. Rocha², A. Gomes², I. Santana², M. Cunha¹, A.F. Jorge², A. Felix³

¹IPO Lisbon - Clinical Pathology Lab. Virology (Portugal), ²IPO Lisbon - Gynecology Serv. (Portugal), ³IPO Lisbon - Anatomy Pathology (Portugal)

Background / Objectives

Vulvar cancer is the fourth most common gynecologic cancer. Regarding invasive squamous cell carcinoma (SCC), two independent pathways have been described: one related with HPV infection affecting young patients and the other in older age with non-neoplastic vulvar epithelial disorders. In Europe, the prevalence of HPV associated with vulvar cancer is 34.7% [1], although a wide geographic variation has been described [2]. Invasive carcinomas harboring HPV DNA have been associated with better prognosis [3,4]. Our aim was to evaluate the prevalence of HPV in a single center in Portugal and to make a correlation with clinical data, namely age of patient at diagnosis and prognosis.

Methods

We evaluated the presence of HPV DNA in 84 women with SCC treated in our institution between 2008 and 2015. HPV DNA was evaluated with an In House assay: after DNA extraction (QIAamp MinElute Virus Kit, QIAGEN), a 75 bp amplicon (SPF10 primers) was amplified using Real Time PCR (SYBR Green dye). Samples with a positive result were genotyped using HPV INNO-LIPA.

Results

Patients median age is 74, ranging from 36 to 92. HPV DNA is present in 26 SCC (31%) being 20 (23%) for HR HPV, 3 (4%) for LR HPV and 3 (4%) for HPV X. The most prevalent genotypes are HPV16 (38.5%) and HPV 39 (12.5%). Regarding Low Risk genotypes, we detected HPV 6 (n=2) and 42 (n=1); 17% (n=4) had multiple infections, mainly with HPV 16 (n=3). The overall survival rate in patients with HPV was 71%; in patients without HPV infection the survival was only 62%.

Conclusion

Our detection of HPV DNA in squamous cell carcinoma of the vulva (31%) is in agreement with the prevalence of HPV in vulvar cancer in European countries

(34.7%). We did not find an association between SCC of vulva with HPV DNA with patient age, and survival.

References

- [1] De Vuyst et al, *Int. J. Cancer*: 124, 1626-1639 (2009)
- [2] Sanjose S et al, *Eur J Cancer* 49:3450-61 (2013)
- [3] Lindell G et al. *Gynecol Oncol* 117: 312–316 (2010).
- [4] Alonso I et al, *Gynecol Oncol* 122: 509-14 (2011).

OC 08-01

THE REVOLUTION IN CERVICAL CANCER DETECTION FROM CONVENTIONAL CYTOLOGY TO REAL-TIME MOLECULAR DETECTION

A. Rabaan

Johns Hopkins Aramco Healthcare (Saudi arabia)

Background / Objectives

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Evidence linking HPV to cervical carcinoma is extensive. High risk HPV (hrHPV) testing is recommended for cervical cancer screening. This is a prospective study that enrolled women of all ages screened for cervical cancer who showed atypical squamous cells of undetermined significance (ASCUS). The study aim to compare the performance of Xpert® HPV assay (Cepheid, Sunnyvale) and Hybrid Capture® 2 (HC2) assay (Qiagen, Gaithersburg, MD) for detection of hrHPV infection in cervical smear samples. Also we determined the prevalence of hrHPV among women in area where no previous studies took place

Methods

Cervical cells, Pap specimens, from 168 subjects were collected with a Cytobrush that was washed into the PreservCyt collection medium (Hologic, Inc., Marlborough, and MA) kept at room temperature waiting for a liquid base cytology conventional cytology. All samples were reviewed by cyto-technologists and the final diagnosis was confirmed by the pathologists. The cervical cancer screening program in our institution states that, routinely, only samples showing atypical squamous cells of undetermined significance (ASCUS) cytology will be referred for hrHPV testing by Hybrid Capture® 2 (HC2) assay (Qiagen, Gaithersburg, MD). All samples tested by the latter were also tested by the Xpert® HPV assay. Both assays were performed in the molecular diagnostic laboratory at Johns Hopkins ARAMCO healthcare between January and July 2014.

Results

A total of 168 ASCUS samples were enrolled in the study. Among them, 134 (79.8%) were from Saudi patients. The mean age + SD were 40.6 + 11.6 years, ranging from 20-83 years. Xpert® HPV assay reported 30 samples as positive (17.6%; 95% CI, 12.6 to 24.1), compared to 36 for the Hybrid Capture® 2 (HC2) assay (21.2%; 95% CI, 15.7- 27.9). The positive rates did not differ statistically among Saudi and

non-Saudi patients. The overall concordance rate between the two tests was 98.2% with a positive concordance rate of 91%. There were three samples that tested positive on Hybrid Capture® 2 (HC2) assay were reported negative by Xpert® HPV assay. HPV others types, HPV 16 and HPV 18/45 types constituted the most common types in a decreasing order

Conclusion

Positive concordance was reasonable between the Xpert® HPV and Hybrid Capture® 2 (HC2) assays. Further investigation is needed to resolve discrepant cases. Overall, the Xpert® HPV assay has a faster turnaround time in comparison with conventional cytology and Hybrid Capture® 2 (HC2) and can be used as a point of care method to detect hrHPV on ASC-US samples.

OC 08-02

PERFORMANCE OF HIGH-RISK HPV DNA
GENOTYPING FOR PRIMARY CERVICAL CANCER
SCREENING AND TRIAGE OF HPV-POSITIVE WOMEN,
COMPARED TO CYTOLOGY. RESULTS OF THE PIPAVIR
STUDY

K. Chatzistamatiou¹, **T. Agorastos**¹, **T. Moysiadis**², **E. Angelis**³, **I. Lekka**⁴, **A. Kaufmann**⁵, **E. Boschetti-Grützmacher**⁵, **A. Krings**⁵, **I. Hagemann**⁶, **I. Koch**⁷, **O. Boecher**⁷, **E. Soutschek**⁷, **A. Skenderi**⁸, **S. Angelidou**⁹, **E. Katsiki**⁹, **N. Maglaveras**⁴, **A. Schneider**⁵, **P. Jansen-Duerr**¹⁰

¹4th Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Hippokratio General Hospital, Thessaloniki (Greece), ²Institute of Applied Biosciences, Centre for Research & Technology - Hellas, 57001 Thessaloniki (Greece), ³School of Informatics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki (Greece), ⁴Laboratory of Bioinformatics, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki (Greece), ⁵Department of Gynecology, Charité Campus Benjamin Franklin and Campus Mitte, Berlin (Germany), ⁶MVZ Im Mare. Kiel (Germany), ⁷Mikrogen GmbH, Neuried (Germany), ⁸Laboratory of Cytology, Hippokratio General Hospital, Thessaloniki (Greece), ⁹Department of Histopathology, Hippokratio General Hospital, Thessaloniki (Greece), ¹⁰Research Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck (Austria)

Background / Objectives

As high-risk (HR) HPV types are detected in 99.7% of cervical cancer cases and in most of pre-invasive cases, HPV detection may be an alternative as a screening test for the detection of precancerous cervical lesions¹. In fact, HR-HPV detection is increasingly considered a better method of primary screening than cytology²⁻⁵. The objective of the presented study is to assess the performance of (HR) HPV DNA genotyping as a method of primary cervical cancer screening and triage of HPV positive women to colposcopy compared to liquid-based cytology (LBC) in an urban population of Greek women.

Methods

Between February 2013 and April 2014, 1,519 women aged 30-60 years (mean: 43,8), who attended routine cervical cancer screening at the Family Planning Centre,

Hippokratio Hospital of Thessaloniki, Greece, provided a cervicovaginal sample for the study. The women were recruited according to the protocol of the PIPAVIR study, which aimed to assess the diagnostic accuracy of HPV DNA testing, cytology and E7-protein testing for cervical cancer screening. The substudy presented herein investigates the first two kinds of testing. Cytological evaluation was performed using LBC (ThinPrep® Hologic, Bedford, MA, USA). An aliquot of each sample was used in order to detect HR HPV using HPV Multiplex Genotyping (MPG), a PCR-based technique. Women positive for cytology [atypical squamous cells of undetermined significance (ASC-US) or worse] or HR HPV were referred for colposcopy. Colposcopically-guided biopsies followed by histological assessment of the samples were performed in cases of abnormal colposcopic impression.

Results

Among 1,432 valid tests the prevalence of HR-HPV and HPV 16 and/or 18 was 18.8% and 11.7% respectively. The cytological report was ASCUS or worse in 6.8% of the women tested. Cervical Intraepithelial Neoplasia grade 2 or worse (CIN2+) was detected in 21 women (1.5%). Sensitivity of cytology (ASCUS or worse) and HPV DNA testing for the detection of CIN2+ was 52.4% and 100%, and specificity was 93.8% and 82.3% respectively. The Positive and Negative Predictive Value (PPV and NPV) were 11.2% and 99.2% for cytology and 7.8% and 100% for HPV testing respectively. HPV 16/18 genotyping presented better sensitivity than cytology concerning triage of HPV positive women to colposcopy (61.9% vs 52.4% respectively), but worse PPV (7.7% vs 11.2% respectively).

Conclusion

For women older than 30 years, HR HPV full genotyping could represent an alternative methodology for primary cervical screening as well as for triage of HPV positive women to colposcopy, in comparison to HR HPV DNA primary screening combined with cytological triage of HPV positive women.

References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*. 1999; 189:12–19. PMID: 10451482
2. Bulkman NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int J Cancer*. 2004; 110: 94–101. PMID: 15054873
3. Castle PE, Stoler MH, Wright TC Jr, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol*. 2011; 12: 880–890. doi: 10.1016/S1470-2045(11)70188-7 PMID: 21865084

4. Agorastos T, Chatzistamatiou K, Katsamagkas T, Koliopoulos G, Daponte A, Constantinidis T, Constantinidis TC; HERMES study group. Primary screening for cervical cancer based on high-risk human papillomavirus (HPV) detection and HPV 16 and HPV 18 genotyping, in comparison to cytology. *PLoS One*. 2015 Mar 20;10(3):e0119755. doi: 10.1371/journal.pone.0119755. eCollection 2015.

5. Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*. 2014; 383: 524–532. doi: 10.1016/S0140-6736(13)62218-7 PMID: 24192252

OC 08-03

FIRST ROUND OF CO-TESTING IN THE AREA OF HOSPITAL DE BARBASTRO (SPAIN)

R. Oncins, M.D. Comes, M.A. Aragón, E. Clemente, V. Víctor, M. Gorka, G. Lorena

Hospital de Barbastro (Spain)

Background / Objectives

To describe the first round of cervical cancer screening in the Barbastro area, where co-testing was conducted, and to study the effectivity and sensitivity of the HPV-DNA testing.

Methods

CIN2+ cases diagnosed between January 1st, 2011 to December 31st, 2015 and follow-up until January 31st, 2016; were described. The target population was 24,501 women between 30 to 64 years. Patients came from primary care screening and gynaecological consultations. We screened with hrHPV DNA testing and cytology according to SEGO 2010 protocols, which recommend screening with co-testing every 5 years.

HC2® (Qiagen) was the assay used during the first 10 months giving positive or negative results. Cobas® 4800 (Roche) was used during the remaining period giving the genotype for HPV 16, 18 and the pool of 12 hrHPV (31, 33, 35, 39, 45, 51 52 56, 58, 59, 66 y 68).

Loop electrosurgical procedure (LEEP) was the first choice to treat cervical intraepithelial neoplasia (CIN). Repeated conisation and hysterectomy with or without anexectomy followed initial treatment when necessary. Co-testing at 6 months and follow-up with HPV-DNA testing y/or cytology at 12 and 24 months were recommended.

Results

A total of 238 women with an age average of 37.9 ± 10.3 participated in the clinical program; 162 (68.1%) were born in Spain and 65 (27.3%) overseas. 37 women (15.5%) had follow-up after having positive HPV test with negative cytology results. 57 (25.4%) patients were positive for HPV 16, 7 (3.1%) for HPV 18, and 74 (33.0%) tested positive for the pool of hrHPV. 4 (1.8%) women tested negative: 2 women

presented a CIN2/3 and 2 presented invasive carcinoma (one adenocarcinoma and one squamous carcinoma) with Quiagen and/or Roche test. Sensitivity was 77.9% and 98.7% for Cytology and HPV testing, respectively.

18 women had invasive carcinomas, 7 of which were microinvasive (38,9%). Out of the 220 CIN cases, 37 (15.5%) were CIN2 and 183 (76.9%) were CIN3. The horizontal extension of CIN was less than 1mm in 27 (21.3%). Clear margins after LEEP were obtained in 171 (84.2%) patients. Reoperation was necessary because of residual disease in 8 CIN (3.4%), and all microinvasive carcinomas.

At first post-treatment control, 133 patients (77.3%) were negative for HPV. Positive HPV results persisted in 22 patients (12.6%). A correlation with been born overseas ($p:0.008$), positive margins ($p:0.023$) and horizontal extension less than 1 mm ($p:0.036$) was detected.

Conclusion

Efficiency of co-testing is shown in our area. Persistent HPV positive results after CIN treatment are very low and are associated with been born overseas, positive margins and very focal extensions, suggesting the last a multifocal affectation.

OC 08-04

COST-EFFECTIVENESS ANALYSIS STUDY OF HPV TESTING AS A PRIMARY CERVICAL CANCER SCREENING IN THAILAND

W. Termrungruenglert¹, N. Khemapech¹, T. Tantitamit², S. Sangrajrang³, P. Havanond¹

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok (Thailand), ²Department of Obstetrics and Gynecology, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok (Thailand), ³National Cancer Institute, Bangkok (Thailand)

Background / Objectives

Primary HPV screening test can be considered as an alternative strategy to current cytology-based cervical cancer screening method. It is a cost saving method from several economic studies by reducing number of screening tests and overall screening costs. The aim of this study is to use the model to compare the cost and benefit of different screening strategies involving HPV 16/18 genotyping, compared with hrHPV testing alone or cytology for detecting CIN2+.

Methods

Economical analysis using Markov modelling approach to combine the relevant epidemiological existing data from current population-based study of The National Cancer Institute of Thailand. A cohort of 100,000 hypothetical healthy female population age 30-65 years were simulated in each strategy. The intervention of this study is the management guideline for cervical cancer screening using HPV 16/18 genotyping with reflexed cytology of women aged 30-65 years every five years screening interval vs alternative protocol using hrHPV testing alone or cytology-based screening. The model was based on the assumption of complete screening compliance. The unit costs of screening were modeled from the perspective study of The National Cancer Institute of Thailand and King Chulalongkorn Memorial Hospital. Treatment costs for diseases were not included because of equal occurrence in both groups. The main outcomes were defined as a number of CIN2+ cases and cost per 100,000 women screening over 35 years.

Results

Model predictions indicated that, the most cost-effectiveness strategy is hrHPV testing alone by reducing cost and also increase CIN2+ detection rate. It would turn

an additional 130 cases and decrease cost (cost saving) by 46,950,840 THB (1,173,771 EUR) per 100,000 women screened when compared to HPV 16/18 genotyping. Compare with cytology, hrHPV testing decrease cost (cost saving) by 51,279,781 THB (1,281,995 EUR) and detect more 506 cases of CIN2+. Cytology screening is the least effectiveness and costliest method among 3 strategies. From sensitivity analysis, the cost of HPV testing, cost of colposcopy, incidence of HPV infection and sensitivity of cytology may affect the results. HPV 16/18 genotyping would be the most cost effective method if the cost of colposcopy increase to 3,573 THB (89 EUR) or the incidence of HPV infection increase 3 times or more in this study.

Conclusion

The results of this cost-effective analysis support the full scale implementation of HPV testing as a primary cervical cancer screening.

OC 08-05

CONTRIBUTION OF SCREENING CYTOLOGY TO THE DIAGNOSIS OF INVASIVE CERVICAL CANCER IN THE CONTEXT OF COTESTING EVERY 3 YEARS

W. Kinney¹, **J. Gage**², **B. Fetterman**³, **N. Poitras**³, **M. Schiffman**², **N. Wentzensen**², **T. Lorey**³, **P. Castle**⁴

¹Regional Laboratory, Kaiser Permanente Northern California, Berkeley, CA, USA (United States of America), ²Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, MD, USA (United States of America), ³Regional Laboratory, Kaiser Permanente Northern California, Berkeley, CA, USA; (United States of America), ⁴Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, USA (United States of America)

Background / Objectives

To clarify the contribution of cytology to the earlier diagnosis of invasive cervical cancer as part of screening cotesting every 3 years.

Methods

Women who underwent cotesting within Kaiser Permanente Northern California over the period 2003 through 2013 and subsequently were diagnosed with invasive cervical cancer were identified. Completeness of ascertainment of cancer cases was verified by comparison to the Northern California SEER registry. Laboratory results and clinical records were reviewed to establish the contribution of the screening cytology component of cotesting to the diagnosis of invasive cervical cancer.

Results

From 2003 through 2013, 699 women underwent cotesting at KPNC prior to the diagnosis of invasive cancer. One hundred seventy (24%) of the 699 cancers had at least one HPV-negative cotest at any time interval prior to the diagnosis. Of the 170 women with one or more HPV negative results, 116 (68%) and 39 (23%) were Pap negative and positive, respectively. Fifteen women (9%) had both Pap negative and Pap positive diagnoses. The following clinical settings characterized this heterogeneous group (categories are not mutually exclusive):

Most recent Pap positive HPV negative result > 3 years before cancer diagnosis: 7

Probable Stage 2 Adenocarcinoma of the endometrium: 5

Cancer biopsy before results of cotest returned: 9

Symptomatic from cancer or clinically apparent cancer on exam at testing visit: 7

Cotested as part of follow-up of a previous positive HPV test: 3

Subsequent positive HPV test prior to diagnosis: 11

Cotest result was ASC-US HPV negative (for which followup testing in 3 years is recommended): 14

Of the 54 women with HPV-negative, Pap-positive results prior to a cancer diagnosis, a maximum of 14/699 (2%) of women might have had their primary cervical cancer diagnosed earlier because of the inclusion of cytology as part of their screening cotest conducted within 3 years of cancer diagnosis. During the study period, 2,438,474 cotests were performed in KPNC.

Conclusion

The majority of women with HPV-negative cotests prior to a cancer diagnosis were also Pap-negative. Of the 54 women with Pap-positive HPV-negative cotests reported from KPNC prior to the diagnosis of cervical cancer, most were either not screening tests, were associated with cancer likely originating from the endometrium, had ASC-US cytology or had a subsequent positive HPV test prior to the cancer diagnosis. Description of the clinical context of testing is required to accurately characterize the potential contribution of including cytology in screening cotesting to the diagnosis of invasive cervical cancer.

OC 08-06

HIGH-RISK HUMAN PAPILLOMA VIRUS PREVALENCE BY AGE AFTER RANDOMIZED IMPLEMENTATION OF HPV-TEST IN PRIMARY SCREENING

A. Tropé, B. Engsæther, M. Nygård

The Cancer Registry of Norway (Norway)

Background / Objectives

High-risk human papilloma virus (HR-HPV) testing as primary screening for cervical cancer, is currently implemented among women 34-69 years old in four Norwegian counties, counting approximately 285 000 women. Based on available international literature, we predicted an overall HR-HPV prevalence of 8% in this population. Our aim was to study the actual prevalence of HR-HPV prevalence in target age group for the first time in Norway.

Methods

A randomized implementation of HR-HPV testing in primary screening was started February 1st 2015. All three participating laboratories use HPV COBAS 4800 HPV Test (Roche Diagnostics) testing for 14 HR-HPV genotypes (16, 18 and 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), the same biobank solutions, and similar quality assurance protocols. All test results (HR-HPV, cytology and histology) are reported to the Cancer Registry of Norway for administration and surveillance. So far 23 851 women have been tested.

Results

During the first year of primary HPV screening, the overall HR-HPV positivity rate was 6.7%. There were only minor differences in the HPV positivity rates between laboratories. The HR-HPV positivity rate declined as a function of age, with a weak increase in prevalence after 58 years of age. Genotypes 16 and 18 contributed 24% and 16%, respectively, to total HR-HPV prevalence.

Conclusion

HR-HPV prevalence in the age group 34-69 was slightly lower than predicted. There was a big proportion of non 16 and 18 HR-HPV positive women in this population.

The slight increase in HR-HPV positivity among older women and the reasons for this should be explored further.

OC 08-07

HUMAN PAPILLOMAVIRUS TESTING VERSUS LIQUID-BASED CYTOLOGY FOR NON-ATTENDEES OF CERVICAL CANCER SCREENING: RESULTS OF A RANDOMISED CONTROLLED TRIAL

M. Viviano¹, **R. Catarino**¹, **C. Guillot**¹, **I. Royannez-Drevard**¹, **P. Vassilakos**², **P. Petignat**¹

¹Geneva University Hospitals, Gynecology Division, Geneva (Switzerland),

²Geneva Foundation for Medical Education and Research, Geneva, Switzerland (Switzerland)

Background / Objectives

The efficiency of a screening program is significantly influenced by its attendance rate. About 30% of women living in Switzerland do not undergo cervical cancer screening, thus running a higher risk of developing cervical cancer. Our aim was to assess the feasibility and efficacy of HPV self-sampling in reaching women who do not regularly attend cervical cancer screening with the traditional strategy.

Methods

Recruitment took place in Geneva between September 2011 and November 2015. All women between the age of 25 and 69 years, who had not undergone Pap testing in the preceding 3 years, not pregnant and with no prior hysterectomy, were considered eligible to take part in the study. The enrolled participants were then randomized into either the control group, in which they were asked to come to the hospital for a Pap test, or the study group, in which they were invited to perform a home-based HPV self-test. All statistical analyses were done with Stata/IC, Version 14.0.

Results

A total of 667 women were enrolled in the study, of which 336 were randomized into the study group and 331 were assigned to the control group. The mean age of participants was 42.2 ± 10.9 years. Only 218 (32.9 %) women were European, while the majority (n=443, 67.0%) had a foreign nationality. When considering the participants who underwent further clinical management based on their primary screening results, the drop-out rates were 10.4% and 8.8% in the study and in the control group, respectively (p=0.468). The prevalence of cervical intra-epithelial

neoplasia grade 2 or worse (CIN2+) found in the HPV group was 2.9%, comparing to the 1.2% of CIN2+ found in the Pap test group ($p=0.032$). HPV-test positivity to HPV-16 was associated to 60.0% of CIN2+ lesions ($p=0.054$). HPV positivity and ASCUS+ prevalence declined with increasing age.

Conclusion

The HPV self-test is a feasible mean of reaching women who don't regularly attend cervical cancer screening. With a significantly higher sensitivity in detecting CIN2+, this primary screening tool represents a valid substitute to physician-performed Pap testing.

OC 08-08

EVALUATION OF CERVICAL CANCER (CxCa) SCREENING STRATEGIES (CO-TEST, HPV, PAP) USING THE CRMM

C. Popadiuk¹, **C. Nadeau**², **K. Asakawa**², **B. Flanagan**², **S. Memon**³, **C. Gauvreau**³, **A. Coldman**⁴, **S. Gribble**³, **M. Wolfson**⁵, **G. Lockwood**³, **A. Miller**⁶

¹Memorial University (Canada), ²Statistics Canada (Canada), ³Canadian Partnership Against Cancer (Canada), ⁴British Columbia Cancer Research Center (Canada), ⁵University of Ottawa (Canada), ⁶University of Toronto (Canada)

Background / Objectives

Background: In Canada Cervical cytology (Pap) has been the foundation of CxCa screening for over half a century. Primary HPV DNA testing is now replacing it in some provinces whereas in the United States, Pap and HPV testing is used together (co-testing). The Cervix-HPV Cancer Risk Management Model (CRMM) is a Canadian population HPV transmission micro-simulation model that projects impacts of CxCa screening strategies.

Objectives: To assess the health and economic outcomes of 7 CxCa screening strategies in Canada using the Cervix-HPV CRMM.

Methods

Methods: We compared 3 types of screening strategies: 1) Pap only: triennial Pap in ages 21-65 (PAP3); 2) Pap/HPV: triennial Pap in ages 21-29 and HPV DNA every 3 (PAP3/HPV3) or 5 (PAP3/HPV5) years from ages 30-65; and 3) Co-testing: triennial Pap in ages 21-29 and co-testing every 5 years from ages 30-65 with 3 distinct abnormal result follow-up algorithms, (CoT1/2/3), and co-testing every 5 years from ages 21 to 65 (CoT4). We assumed 70% HPV vaccination rate of girls aged 12 starting in 2008 and 80% recruitment rate of age-eligible women, with 70% rescreening. Health system costs and quality-adjusted life-years (QALYs) were discounted at 3%. Costs were in 2008 Canadian dollars.

Results

Results: Compared to PAP3, the PAP3/HPV3 strategy would show the most decline in incidence 15.8%, followed by the 3 strategies CoT1/2/3 (11%), CoT4 (10%), and

PAP3/HPV5 (8%). Similarly, PAP3/HPV3 would show the most decline in deaths (16%), followed by CoT1/2/3/4 (11%) and PAP3/HPV5 (9%). CoT strategies required the most colposcopies in 2050, with between 50% (CoT4) and 88% (CoT1) more than for PAP3, 14% more for PAP3/HPV3 and 10% less for PAP3/HPV5 scenarios. Lifetime costs of vaccination, screening and treatment would be the least with PAP3/HPV5, 5.4% less compared to PAP3. Pap3/HPV3 and CoT1/2/3/4 would be more costly from 17.4%, and 33 to 40% more, respectively. All strategies would have gains in QALYs. Given incremental cost-effectiveness ratios, PAP3/HPV5 would dominate PAP3. Pap3/HPV3 would dominate all CoT strategies, and compared to PAP3/HPV5, would cost \$141,000 per QALY gained.

Conclusion

Conclusions: The Cervix-HPV CRMM projects that in a setting of HPV vaccination, triennial primary HPV DNA testing replacing triennial Pap at age 30 would be more effective than triennial Pap, and more effective and less costly than the co-testing strategies. While extending the screening interval from 3 to 5 years and using HPV DNA testing would be less costly than triennial Pap, it would be less costly and less effective than HPV DNA testing every 3 years. Triennial HPV testing would cost \$141,000 per QALY gained compared to HPV testing every 5 years

OC 08-09

WHEN CAN CERVICAL CANCER BE ERADICATED? A MODEL FOR PROJECTING CERVICAL CANCER INCIDENCE AND MORTALITY FROM 2016 TO 2040

C.R. Cohen ¹, A. Castanon ², R. Landy ², R. Music ¹, P. Sasieni ²

¹Jo's Cervical Cancer Trust (United kingdom), ²Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine (United kingdom)

Background / Objectives

Over the next 25 years, high levels of coverage from the UK's HPV vaccination and Cervical Screening Programmes should result in greatly reduced incidence and mortality from cervical cancer. The Jo's Cervical Cancer Trust model considers the long-term combined effect of both prevention programmes; predicting incidence, staging and mortality from cervical cancer up to 2040 in the UK by age under different scenarios relating to the future of these programmes.

The model is flexible and allows for screening and vaccination rates to be adjusted up or down, predicting the impact on incidence and mortality. It also takes into consideration likely changes to both programmes including the use of a 9-valent vaccine and the introduction of HPV primary screening.

Methods

The Jo's Cervical Cancer Trust model uses data from the audit of cervical cancers in England to estimate (by age) the relative risks of cervical cancer by screening attendance. To allow the underlying cancer incidence to change over the next 25 years in the absence of changes to vaccination/screening, a novel age-period-cohort model was fitted to cervical cancer incidence from 1971 to 2013, and extrapolated over the next 25 years. The model estimates mortality by applying stage- and age-specific excess hazards (from published survival data).

For the baseline scenario we assume that screening is by cytology until 2017, when HPV primary testing is fully implemented. We will discuss three scenarios for future screening coverage (100% coverage, current coverage continues, and no future screening) and three vaccine scenarios (current uptake using the quadrivalent vaccine, and change to 9-valent vaccine in 2018). We assume that both vaccines initially prevent all target HPV types (and for the quadrivalent 15% of other high risk HPV types), but that the efficacy wanes by 0.25% per year.

Results

If screening coverage were to be maintained up to 2040 we would see cervical cancer rates decrease by 60% in women under age 45. For older women, particularly those age 60 and over we would see between 10-20% increases in the rates due to strong cohort effects. In a scenario where screening is slowly phased out we would see a doubling of cervical cancer rates by 2040.

Conclusion

The flexibility of the Jo's Cervical Cancer Trust model allows policy makers to consider the implications of changes to prevention programmes on cervical cancer over the medium- and long-term. Of particular note, with the high vaccination coverage achieved in the UK, the future burden of cancer will shift to older women who have a higher underlying risk of cervical cancer than previous generations.

OC 08-10

Staging Pre-Cervical Cancer Using Combined E6, E7 mRNA Quantification and Cell Cycle Analysis (OncoTect 3Dx)

P. Karakitsos¹, A. Chargin², B. Patterson², K. Shults²

¹National and Kapodistrian University of Athens (Greece), ²IncellDx, Inc, (United States of America)

Background / Objectives

New therapeutics directed at treating pre-cervical cancer changes prior to the development of cervical cancer require staging of the molecular changes associated with transformation and carcinogenesis in order to treat at the earliest possible stage. To that end, we report the preliminary results of a study that uses a single, high throughput assay (OncoTect 3Dx) that defines the stages of squamous cell abnormalities that lead to cervical cancer.

Methods

We analyzed 227 samples that included 79 normals (NILM HRHPV DNA-), 72 low grade (NILM/ASCUS/LSIL HRHPV DNA+), and 76 high grade (HSIL HRHPV DNA+) collected in ThinPrep® liquid-based cytology media. Each sample was assayed using the 96-well OncoTect 3DX assay that quantifies E6, E7 mRNA and cell cycle on a cell by cell basis. In particular, the post-G0/G1% was calculated for each sample as a measure of cell proliferation. In addition, mean corpuscular volume (MCV) was determined for every cell in all samples.

Results

There was an inverse correlation between cervical abnormality stage, normal-low grade-high grade, and MCV with normal samples being 161 uM3, low-grade 131 uM3, and high grade 113 uM3 (Mann-Whitney $P < 0.001$). The post-G0/G1% also differed depending on the stage of abnormality with normal samples and high grade samples having the highest proliferation rate and low grade abnormalities having the lowest proliferation rate (Mann-Whitney $P = 0.03$).

Conclusion

Using multiple parameters quantified using the OncoTect 3DX assay, we were able to define normal cervical samples as E6, E7 mRNA-, MCV hi, post-G0/G1% hi; low grade cervical samples as E6, E7 mRNA +/-, MCV intermediate, post-G0/G1% low;

and high grade cervical samples as E6, E7 mRNA +, MCV low, post-G0/G1% high. The ability to stratify cervical cancer abnormalities in an automated, high-throughput manner is advantageous for companion diagnostic applications.

OC 08-11

HPV E7 ONCOPROTEIN-BASED ELISA ASSAY FOR TRIAGE OF HPV-POSITIVELY SCREENED WOMEN

T. Agorastos ¹, K. Chatzistamatiou ¹, I. Hagemann ², E. Boschetti ³, O. Boecher ⁴, I. Koch ⁴, E. Soutschek ⁴, I. Lekka ⁵, A. Skenderi ⁶, E. Aggelis ⁶, H. Pircher ⁷, P. Jansen-Duerr ⁷, A.M. Kaufmann ³

¹4th Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Hippokratio General Hospital, Thessaloniki, Greece (Greece), ²MVZ Im Mare, Kiel (Germany), ³Clinic for Gynecology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin (Germany), ⁴Mikrogen GmbH, Munich (Germany), ⁵Laboratory of Bioinformatics, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki (Greece), ⁶Laboratory of Cytology, Hippokratio General Hospital, Thessaloniki (Greece), ⁷Research Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck (Austria)

Background / Objectives

Persistent infection of papillomaviruses (PIPAVIR) high-risk (hr) types is a prerequisite for the development of cervical dysplasia. Expression of oncoproteins E6 and E7 and their upregulation during progression is associated with loss of cell cycle control and cellular neoplastic transformation. New high-affinity rabMab antibodies have been developed for detection of E7 of 12 most prevalent hr-HPV types in an ELISA format. Primary HPV screening by highly sensitive methods detecting HPV nucleic acids needs efficient triaging. A molecular test detecting hrE7 with comparable specificity to cytology could be advantageous. A clinical trial was performed to generate first data on applicability and sensitivity/specificity of a hrE7-based ELISA.

Methods

PreserveCyte cervical samples (1258) were collected. Cytological samples were prepared and all women got expert colposcopy and biopsy if indicated. Multiplexed genotyping was performed. From the same smear hrE7 ELISA was performed. Different hrE7 ELISA formats were used detecting HPV 16/18/31/33/35/39/45/51/52/56/58/59 (recomWell HPV HR screen); HPV16/31/33/35/52/58 (recomWell HPV plus); or HPV16/18/45 (recomWell HPV 16/18/45) and results correlated to histological findings. Thresholds, sensitivity, specificity, PPV, NPV for detection of CIN2+ were calculated and compared against HPV test and cytology.

Results

HPV testing by MPG had by definition a sensitivity of 100% (25/25) in detecting CIN2+ in a triage setting considering all hr-HPV positives (n=269). PPV was only 9.29%. Sensitivity of cytology as triage for HPV-positive women to colposcopy was 52% (13/25) and specificity 85% (208/244), PPV was 26.5% and NPV 94.5%. hrE7 ELISA as triage was performed in the different formats. Detection by recomWell HPV HR screen, recomWell HPV plus, and recomWell HPV 16/18/45 had sensitivity of 92%, 76%, 72%; a specificity of 43%, 72%, 71%; a PPV of 14.2%, 22.1%, 20.5, and NPV of 98.1%, 96.7%, 96.1%, respectively.

Conclusion

A molecular triage of hrHPV positive screening results would have technical advantage over PAP cytological triage and could be performed as reflex testing, especially on HPV16 or 18 positive women. Triage of hrHPV DNA positive women for detection of CIN2+ by cytology has slightly better performance than E7 testing in terms of PPV. hrE7 ELISA testing showed higher sensitivity than cytology. RecomWell HPV plus had similar PPV and sensitivity as cytology. All hrE7-ELISA formats have shown higher sensitivity and comparable specificity, PPV and NPV as compared to cytology in detection of CIN2+ dysplasia. Therefore, the new hrE7-ELISA could be viewed as an alternative test to cytology in triaging hrHPV positively tested women.

OC 09-01

RISK STRATIFICATION OF HIGH-RISK HUMAN PAPILLOMAVIRUS POSITIVE WOMEN: IMPACT OF CYTOLOGY AND HPV16/18 GENOTYPING

L.T. Thomsen ¹, C. Dehlendorff ², C. Munk ¹, J. Junge ³, T. Iftner ⁴, K. Frederiksen ², S.K. Kjaer ¹

¹Unit of Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen (Denmark), ²Unit of Statistics, Bioinformatics and Registry, Danish Cancer Society Research Center, Copenhagen (Denmark), ³Department of Pathology, Hvidovre Hospital, University of Copenhagen, Hvidovre (Denmark), ⁴Section Experimental Virology, University Hospital of Tübingen, Tübingen (Germany)

Background / Objectives

Cervical cancer screening using primary human papillomavirus (HPV) testing requires triage of high-risk HPV (hrHPV) positive women. In a large prospective cohort, we investigated the 5-year risk stratification for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) provided by three potential triage strategies: 1) HPV16/18 genotyping; 2) Cytology; 3) Cytology followed by HPV16/18 genotyping.

Methods

Residual liquid-based cytology samples (SurePath) were collected from 40,399 women screened for cervical cancer in Copenhagen, Denmark, during 2002–2005. Samples were HPV tested with Hybrid Capture 2, and positive samples were genotyped with INNO-LiPA. The cohort was followed until February 2015 in a nationwide pathology register for development of CIN3+.

Results

Among hrHPV positive women aged 30–64 years (n=3,386), the 5-year risks of CIN3+ in each triage scenario were:

- 1) HPV16/18 triage: 25.0% (95% CI, 22.2–27.8) in women with HPV16/18 versus 6.5% (95% CI, 5.5–7.5) in those with non-HPV16/18 hrHPV.
- 2) Cytology triage: 39.5% (95% CI, 35.6–43.4) in women with ASC-US+ versus 5.4% (95% CI, 4.5–6.3) in those with normal cytology.
- 3) Cytology followed by HPV16/18: 39.5% (95% CI, 35.6–43.4) in women with ASC-

US+; 11.8% (95% CI, 9.3–14.2) in those with normal cytology and HPV16/18; and 3.2% (95% CI, 2.4–4.0) in those with normal cytology and non-HPV16/18 hrHPV.

Conclusion

HrHPV positive women with HPV16/18 or ASC-US+ have CIN3+-risks above commonly accepted thresholds for immediate colposcopy referral. Women with normal cytology and non-HPV16/18 hrHPV have a non-negligible 5-year risk for CIN3+ (3.2%).

OC 09-02

BAYESIAN ANALYSIS OF BASELINE RISK OF CIN2+ AND CIN3+ BY HPV GENOTYPE IN A SCREENING COHORT OF NILM AND ASCUS SUBJECTS

T. Wright¹, V. Parvu², M. Stoler³, C. Cooper², S. Kodsi², K. Yanson²

¹Department of Pathology and Cell Biology, Columbia University, New York, NY (United States of America), ²BD Diagnostics, Sparks, MD (United States of America), ³Department of Pathology, University of Virginia, Charlottesville, VA (United States of America)

Background / Objectives

HPV primary screening is being implemented in a number of countries around the world. However the optimal approach to determine which HPV positive women require colposcopy and which can safely be retested at some interval is unclear. In some countries genotyping for a select group of high-risk HPV types, either alone or in combination with cytology is being used. It is well known that genotype 16 presents the highest risk of significant disease, but genotyping for other specific high-risk genotypes may also be beneficial. A key piece in determining the genotypes to test is understanding the risk of cervical disease by individual genotype in a screening population. Subjects with high enough risk of CIN3+ should be referred to colposcopy, while those at lower risk may be retested at some interval to allow an opportunity for regression to occur. Determining exact risk thresholds is a subjective endeavor, and should be left to individual guideline groups. Informed decisions will require sufficiently strong evidence to stratify the risk of disease among the high-risk HPV types.

Methods

The population in this study is a subset of subjects enrolled from a screening population in an ongoing US registration trial. The subset includes Hybrid Capture 2 (HC2) (Qiagen, Germantown, MD) positive subjects with ASCUS (≥ 21 years) or NILM (≥ 30 years) cytology ($n = 1933$). These subjects underwent colposcopy and a standardized biopsy protocol. All biopsies had consensus panel review blinded to cytology and HPV status. For this substudy, specimens from subjects diagnosed with CIN2+ and a random selection of <CIN2 subjects were sequenced. The method included PCR amplification, denaturing high performance liquid chromatography separation and bi-directional Sanger sequencing (Transgenomic, Inc., Omaha, NE) to determine specific HPV genotype(s) ($n = 921$ subjects with HPV genotyping and histological diagnosis).

Results

Analysis is ongoing, but we will present baseline risk of disease by HPV genotype analyzed using a multivariate Bayesian model. The risks of CIN2+ and CIN3+ will be calculated with 95% credible intervals. In addition, the probability that the baseline risk of disease from one genotype is greater than the baseline risk of disease from another genotype will be derived by Markov-Chain Monte Carlo (MCMC) methods.

Conclusion

The Bayesian multivariate model is a novel way of estimating the risk of disease by genotype, with intuitive treatment of subjects with multiple infections. This method also allows for a clear quantification of the risk ordering between two genotypes: the probability that the risk of CIN3+ for one genotype is greater than the risk of CIN3+ for another genotype.

OC 09-03

DETERMINATION OF THE 5-YEAR LONGITUDINAL NEGATIVE PREDICTIVE VALUE OF THE APTIMA HPV TEST IN A ROUTINE SCREENING POPULATION IN GERMANY

T. Iftner¹, S. Becker², K.J. Neis³, A. Castanon⁴, A. Staebler⁵, M. Henes², C.H. Von Weyhern⁵, S. Brucker², P. Sasieni⁴

¹Division of Experimental Virology, Institute of Medical Virology, University Hospital Tübingen (Germany), ²Department of Gynecology and Obstetrics, University Hospital Tübingen (Germany), ³Frauenärzte am Staden, Saarbrücken (Germany), ⁴Centre for Cancer Prevention, Queen Mary University of London (United Kingdom), ⁵Department of Pathology and Neuropathology, University Hospital Tübingen (Germany)

Background / Objectives

To date there are no data available on the 5-year longitudinal clinical performance of the RNA-based Aptima HPV test (AHPV) in screening populations. Thus, we compared AHPV and HC2 performances to liquid-based cytology (LBC) in women aged 30–65 attending routine cervical screening and follow both test-positive and -negative women for up to 5 years.

Methods

Women (N=10,040) were screened at office-based gynaecologists. All specimens were collected and tested centrally by LBC, AHPV and HC2. Women were referred to colposcopy if they had an abnormal cytology result and/or were tested positive on either HPV assay. Sensitivity, specificity and positive predictive values were calculated based on review histology. For follow-up, 482 women with a positive test result and no treatment were followed up for 5 years with annual testing. In addition, 5 years after baseline, cervical samples are being collected from a random sample of 4000 study participants who tested triple negative at baseline for determination of the longitudinal negative predictive value (NPV) and HPV related disease after a 5 year period.

Results

Cross-sectional results of 9336 women have recently been published and show comparable sensitivities for CIN2+ and CIN3+ detection, while the positive predictive

value (PPV) and specificity for <CIN2+ was significantly higher for the AHPV test compared to HC2. An interim analysis of the 48 months follow-up data shows a cumulative proportion of HPV clearance at month 28 of 78%. With regard to 15 incident CIN3+ lesions during follow up we calculated a PPV of the HC2 and AHPV base line test result of 3.9% (2.0-6.8) vs 5.0% (2.6-8.6), respectively, while the number of women requiring follow up deduced from the base line result is lower for women tested by the AHPV test. Furthermore, preliminary data from 10% of the target population of the 5-year follow-up cohort of triple-negative women suggest a similar longitudinal NPV of AHPV and HC2. More robust data will be presented at the conference.

Conclusion

In conclusion, our data demonstrate a non-inferior performance of the RNA-based AHPV test in comparison to the gold-standard DNA-based HC2 test.

OC 09-04

HPV BASED SCREENING OF 1.2 MILLION WOMEN AND MEGA HPV LABORATORY PROCESSING IN TURKEY

M. Gultekin, I. Kucukyildiz

no (Turkey)

Background / Objectives

HPV based screening had begun to be recommended for most of the countries in the world, based on the recent scientific evidence (1-3). It had been implemented to the Turkish cervical cancer screening program in 2014. Turkish data is now the largest prospective series on primary HPV screening in the world. The program is organised and all ladies over 30 years of age are screened for 13 High Risk HPV types via HC2. The ones with positive results are further analyzed after DNA extraction for HPV genotypes (Genomica) for epidemiological purposes together with reflex cytology evaluations. All analysis is done in a central MEGA HPV laboratory implemented in Ankara.

Methods

The program is organised and all ladies over 30 years of age are screened for 13 High Risk HPV types via HC2. The ones with positive results are further analyzed after DNA extraction for HPV genotypes (Genomica) for epidemiological purposes together with reflex cytology evaluations. All analysis is done in a central MEGA HPV laboratory implemented in Ankara. Daily, >5,000 samples are analyzed and the results are reported within 10 days of collecting the sample. A specialized population based software is used to evaluate and monitor all steps from sample collection and transport to reporting results, showing the progress through each step of this 10 day process. Untill now, almost 1.2 million ladies have been screened with HPV DNA testing and no samples have been lost or mixed up

Results

A total amount of 1.152.242 ladies had been screened by the end of January 2016 since August 2014. Nearly 100.000 ladies are being screened monthly and by the mid of the year it will be over 1.5 million. The overall HPV positivity rate is 3.58%. The most common genotypes were 16 (27,6%) and 18 (7,75%).The cytoanomaly rate is around 20% among HPV positive cases. The most common

anomalies detected were LGSIL (11,9%), ASC-US (6,09%), HGSIL(0,78%), ASC-H (0,21%) and AGC(0,45%)

Conclusion

HPV Prevalence was found to be very low in Turkey which makes this test a good screening method for us. HPV screening has increased number of people attending to the screening program. The software also gives a national HPV Map, with genotypes across the whole country for epidemiological monitoring. This is a unique and a first nationally organised and implemented HPV based screening program in the world.

References

1. Huh WK, et. al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. *Gynecol Oncol* 2015 Feb;136(2):178-82.
2. Comprehensive cervical cancer control: a guide to essential practice – 2nd ed. World Health Organization publications. 2014, Geneva.
3. Arbyn M, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. *Am J Obstet Gynecol*. 2013 Mar;208(3):184.e1-184.e11.

OC 09-05

PREVALENCE OF HPV AND CYTOLOGIC ABNORMALITIES IN THE BD HPV ONCLARITY STUDY

M. Stoler¹, K. Yanson², T. Wright³, V. Parvu², C. Cooper², S. Kodsi², K. Eckert²

¹Department of Pathology, University of Virginia, Charlottesville, VA (United States of America), ²BD Diagnostics, Sparks, MD (United States of America), ³Department of Pathology and Cell Biology, Columbia University, New York, NY (United States of America)

Background / Objectives

Policy decisions on the age to initiate cervical cancer screening and screening methods are driven in large part by the prevalence of high-risk HPV (hrHPV) infection, cytologic abnormalities, and biopsy-confirmed CIN 2+ in the target screening populations. The distribution of these factors will depend in part on demographics and HPV vaccination status. The BD HPV Onclarity study enrolled 33,858 women 21-83 years presenting for routine cervical cancer screening in the U.S. between August 2013-June 2015. This study provides an opportunity to better understand the prevalence of hrHPV infections and cytologic abnormalities in the U.S.

Methods

The BD HPV Onclarity study enrolled women undergoing routine screening at 31 collection sites in 18 states in the U.S. At enrollment, women had a gynecologic examination that included collection of a SurePath cytology specimen that was processed for cervical cytology and also tested for high-risk HPV genotypes using the BD HPV Onclarity assay. This assay provides genotyping information for HPV 16, 18, 31, 33/58, 35/39/68, 45, 51, 52, 56/59/66. In addition, a second cervical specimen was collected into a ThinPrep vial and was tested using Hybrid Capture 2 (HC2). US laboratories performed cytology testing (n=3) and HPV testing (n=4). Subjects with positive cytology (>ASC-US), or a positive HPV test, or unsatisfactory cytology, as well as a random sample of women negative on cytology and HPV were assigned to colposcopy which followed a standardized biopsy protocol. All biopsies underwent consensus panel review that was blinded to cytology and HPV status.

Results

The median age of the subjects was 37.0 years. Race/Ethnicity data showed 19.4% Hispanic, 1.4% Asian, 18.9% Black, and 78.1% White. Vaccination of enrollees was capped at 10%. 14.2% of the subjects had an abnormal Pap in the last 3 years and 8.9% had a colposcopy within the last 5 years. The prevalence of cytologic abnormalities and HPV positivity by age is shown below.

	21-29 yrs	30-39 yrs	>40 yrs	Overall
NILM	86.2%	91.3%	93.1%	90.6%
ASC-US	7.8%	5.5%	4.8%	5.8%
>ASC-US	5.8%	3.1%	1.9%	3.3%
Unsat	0.2%	0.1%	0.2%	0.2%
HC2 (+)	24.0%	12.9%	7.8%	13.8%
BD Onclarity Assay (+)	24.8%	13.1%	8.0%	14.1%

Conclusion

The BD HPV Onclarity study offers a unique opportunity to better understand factors such as prevalence of high-risk HPV (hrHPV) infection, cytologic abnormalities, and biopsy-confirmed CIN 2+ in U.S. target cervical cancer screening populations. This information will aid in policy decisions on age to initiate cervical cancer screening and screening methods.

OC 09-06

PRELIMINARY 48 MONTH EXIT RESULTS FROM THE HPV FOCAL CERVICAL CANCER SCREENING TRIAL

A. Coldman¹, D. Van Niekirk², M. Kraiden³, L. Smith², D. Cook³, L. Gondara², R. Martin⁴, S. Gavin⁴, D. Quinlan⁴, M. Lee⁴, K. Ceballos², S. Peacock¹, E. Franco⁵, G. Ogilvie⁴

¹BC Cancer Research Centre (Canada), ²BC Cancer Agency (Canada), ³BC Centre for Disease Control (Canada), ⁴University of British Columbia (Canada), ⁵McGill University (Canada)

Background / Objectives

HPV FOCAL is a RCT conducted within the organized cervical cancer screening program in British Columbia (BC) Canada. HPV FOCAL is comparing

the efficacy of high-risk HPV DNA testing, with Liquid Based Cytology (LBC) triage for HPV positives, to LBC testing with HPV triage for ASCUS,

for the detection of >CIN3 over 48 months. Presented are age stratified preliminary CIN detection rates at the 48 month exit.

Methods

Over 18,000 women aged 25-65 were randomized into the Control and Intervention arms. Intervention Arm (IA): Baseline HPV testing,

if HPV negative (HPV-) exit at 48 months with both HPV and LBC (co-testing).

Control Arm (CA): Baseline LBC testing, if LBC negative (NILM),

rescreened at 24 months with LBC and exit at 48 months with co-testing. This analysis includes 48 month exit data for women randomized

before July 1, 2011 with baseline screen negative (HPV- or NILM) results. Co-test results and detection rates of \geq CIN2 and \geq CIN3 are reported.

Results

There were 13091 women (6538 Control and 6553 Intervention) randomized by July 1, 2011 with baseline negative results.

The exit co-test positivity rate was 6.0% for Control versus 4.6% for the Intervention (P<.001) arm. For women of all ages,

the >CIN2 rate was significantly higher in the Control than the Intervention arm (CA: 7.0/1000 [95%CI: 5.2, 9.4] vs.

IA: 3.4 [95% CI: 2.1, 5.1], p-value 0.003). The CIN3 rate was also higher in the Control arm but not significant

(CA: 2.9 [95%CI: 1.8, 4.5] vs. IA: 1.4 [95%CI: 0.6, 2.6] p-value 0.06). The most >CIN2 detected in either arm was in women

25-29yrs at baseline (CA 32.2/1000 vs. 1A 14.7/1000), the lowest >50yrs (CA 3.7 vs IA 1.7). Across all ages, the

highest >CIN2 and CIN3 rates were in women HPV+ but LBC- at 48 months.

Conclusion

Four years after initial testing, more >CIN2 was identified in women who screened LBC negative at baseline than HPV negative,

illustrating the safety of the extended interval with a negative HPV result. The highest >CIN2 and CIN3 exit findings for women

of any age were in those with HPV+/NILM results, demonstrating that HPV, not cytology results are predictive of dysplasia.

These findings are highly informative for programs planning for HPV-based testing with an extended screening interval of 4 years.

OC 09-07

CERVICAL SCREENING WITH AN INTERVAL BEYOND FIVE YEARS REQUIRES DIFFERENT RESCREEN TIMING FOR HPV-NEGATIVE AND HPV-POSITIVE, TRIAGE NEGATIVE WOMEN: FOURTEEN YEARS FOLLOW-UP OF THE DUTCH POBASCAM TRIAL

M. Van Zummeren ¹, M.G. Dijkstra ², L. Rozendaal ¹, F.J. Van Kemenade ³, T.J.M. Helmerhorst ⁴, P.J.F. Snijders ¹, C.J.L. Meijer ¹, J. Berkhof ⁵

¹Department of Pathology, VU University Medical Center, Amsterdam (Netherlands), ²Department of Obstetrics and Gynaecology, VU University Medical Center, Amsterdam (Netherlands), ³Department of Pathology, Erasmus MC, Rotterdam (Netherlands), ⁴Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam (Netherlands), ⁵Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam (Netherlands)

Background / Objectives

HPV-based cervical screening programmes with a screening interval beyond five years, as currently implemented in the Netherlands, must be evaluated and supported by estimates of the long-term cancer and CIN3 risks.

Methods

Fourteen years follow-up data including three consecutive five-yearly screening rounds of a Dutch population-based randomized screening cohort* were collected from women with a negative HPV DNA and/or negative cytology test (n=43,339, aged 29-61 years). At baseline, women were randomly assigned to HPV DNA and cytology co-testing (intervention) or cytology testing only (control) and managed accordingly. In the control group, HPV DNA test results were blinded. Cumulative cancer and pre-cancer (CIN3+) incidences were calculated by Kaplan-Meier methods among HPV DNA negative, cytology negative, and among women with a single or double negative test. In HPV DNA positive, cytology negative women, we examined whether CIN3+ risk could be further reduced by HPV16/18 genotyping and repeat cytology.

Results

The cumulative cancer and CIN3+ incidence among HPV DNA negative women in the intervention group, after three rounds of screening (0.09% and 0.6%) were similar to the corresponding cumulative incidences among women with negative cytology in

the control group after two rounds (0.09% and 0.7%). Furthermore, in the intervention group, the CIN3+ incidence rates among HPV DNA positive women with negative triage were substantially higher than the CIN3+ incidence among HPV DNA negative women (rate ratios 10.4 to 29.1).

Conclusion

The long-term CIN3+ and cancer risks among HPV DNA negative women are low and are supportive of an extension of the screening interval. HPV DNA positive, triage-test negative women have a non-negligible long-term CIN3+ risk and should therefore be rescreened within five years. HPV-based programmes with long intervals can therefore only be implemented in conjunction with risk-stratification.

References

* the POBASCAM trial, trial registration: ISRCTN20781131

OC 09-08

EVALUATION OF P16/KI-67 DUAL STAIN AND HPV16/18 GENOTYPING IN A LARGE POPULATION OF HPV-POSITIVE WOMEN

W. Kinney¹, B. Fetterman¹, R. Bremer², P. Castle³, D. Tokugawa¹, N. Poitras¹, E. Hosfield¹, E. Marlowe¹, T. Lorey¹, M. Schiffman², N. Wentzensen²

¹Kaiser Permanente Regional Laboratory (United States of America), ²National Cancer Institute (United States of America), ³Albert Einstein College of Medicine (United States of America)

Background / Objectives

Cervical cancer screening trials and observational studies have demonstrated that HPV testing provides high negative predictive value, which allows safely extending screening intervals for HPV-negative women. The challenge lies in discriminating between transient HPV infections and prevalent precancers in HPV-positive women. An implementation study was conducted at Kaiser Permanente Northern California (KPNC) to evaluate two candidate triage strategies, p16/Ki-67 dual stain and HPV16/18 genotyping.

Methods

Over 13,000 HPV-positive women who participated in cervical cancer screening at KPNC were enrolled from 2015 to 2016. All women were tested for HPV using the hybrid capture 2 assay and had Pap results based on Surepath liquid based cytology. p16/Ki-67 dual stain was performed on residual Surepath specimens using the CINtec PLUS assay. HPV16/18 genotyping was based on the cobas assay. All testing was implemented and conducted at KPNC. Baseline results for 7,124 women are reported. Since HPV-positive, cytology-negative women have a repeat co-test after 12 months before referral to colposcopy, detection of CIN3 is currently only evaluated for dual stain and HPV16/18 as repeat cotest results are not yet available.

Results

Among all 7,124 HPV-positive women, 4,107 (57.7%) were cytology-positive (ASC-US or higher), 3056 (42.9%) were dual stain positive, and 1406 (19.7%) were positive for HPV16 or HPV18. Among all 3,017 HPV-positive, cytology-negative women, 911 (30.2%) were dual stain positive, and 508 (16.8%) were positive for HPV16 or HPV18. Of 315 CIN3 detected so far, 280 (88.9%) were dual stain positive and 176

(55.9%) were positive for HPV16 or HPV18. Additional disease ascertainment, especially among HPV-positive, cytology-negative women, is still ongoing.

Conclusion

At KPNC, primary HPV screening and dual stain triage of HPV-positives would reduce colposcopy referral compared to HPV-cytology co-testing while achieving high sensitivity. While HPV16/18 testing would reduce colposcopy referral in comparison to dual stain testing, almost half of the prevalent CIN3s would be missed. Additional follow-up is required to evaluate the programmatic performance of both approaches, assessing disease detection and number of colposcopies combining baseline and 1-year repeat co-tests.

OC 09-09

LOW SENSITIVITY OF HC2 FOR CANCER DETECTION IN OLDER WOMEN IN THE ARTISTIC COHORT

C. Gilham¹, A. Sargent², H. Kitchener³, J. Peto¹

¹London School of Hygiene and Tropical Medicine (United kingdom), ²Central Manchester University NHS Foundation Trust (United kingdom), ³University of Manchester (United kingdom)

Background / Objectives

The ARTISTIC cohort is one of the few population-based cohorts with long follow-up for HPV associated cancers.

Methods

The ARTISTIC trial recruited 24,510 women aged 20-64 undergoing routine cervical screening in Manchester in 2001-2004. Women underwent LBC and HC2 testing over 3 screening rounds. The women have been flagged for cancer incidence and mortality through national registrations with up to 14 years follow-up. Standard Incidence Ratios (SIR, compared to national cancer incidence rates) and rate ratios (RR) using Poisson regression were calculated.

Results

As expected, the incidence rate of cervical carcinoma-in-situ (CIS) was slightly higher than the national rate in this screened cohort (n=388, SIR=1.2, 95% CI: 1.1-1.3), and the incidence of cervical cancer was lower (n=23, SIR=0.6, 95% CI: 0.4-0.9). The ratio of CIS to cervical cancers decreased sharply with age. The ratio was 130:1 in women aged under 30 years (259 CIS:2 cancers), 12:1 in women aged 35-39 (55 CIS:10 cancers), 6:1 in women aged 40-49 (55 CIS:10 cancers), 3:1 in women aged 50-59 (10 CIS:4 cancers) and 1:1 in women aged over 60 (2 CIS:2 cancers). Among women who developed cervical cancer, all tests were HC2 negative in 2 of 17 women aged under 50 years and 3 of 6 women aged over 50 at diagnosis.

Higher rates of CIS and cervical cancer were seen in those HR-HPV positive at entry, particularly those who were infected with HPV16. There were few vulva, vaginal and anal cancers in the cohort (n=5, 1 & 2 respectively), but the rates were elevated amongst those with HPV16 infection (RR=19.3, 95%CI: 3.3-113.3, p=0.001). There was no excess of head & neck cancers among those HPV positive at baseline.

Conclusion

Despite small numbers of cancers in this screened cohort, there was an elevated risk of vulva, vaginal and anal cancers associated with cervical HPV16 infection. We found no association between cervical HPV infection and head and neck cancer incidence. CIS became much rarer in women aged over 40 and cervical cancer was as common as CIS in women aged over 60 years in this screened cohort. The failure to detect HR-HPV in cancers particularly in older women suggests that either a more sensitive HPV test or improved sampling would be appropriate in women aged over 50.

OC 09-10

INTRODUCING CAREHPV INTO A PUBLIC SECTOR SCREENING PROGRAM IN EL SALVADOR

M. Cremer¹, **M. Maza**², **K. Alfaro**², **J. Felix**³, **J. Gage**⁴, **P. Castle**⁵, **J. Kim**⁶

¹Obstetrics, Gynecology, & Women's Health Institute, Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio; Basic Health International, Colonia las Mercedes, Avenida los Espliegos #5, San Salvador, El Salvador (United States of America), ²Basic Health International, Colonia las Mercedes, Avenida los Espliegos #5, San Salvador, El Salvador (El salvador), ³Department of Pathology, University of Southern California, 2011 Zonal Ave, Los Angeles, California (United States of America), ⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Rockville, Maryland (United States of America), ⁵Albert Einstein College of Medicine, Bronx, New York; Global Coalition Against Cervical Cancer, 100 Radcliffe Drive, Chestertown, Maryland (United States of America), ⁶Center for Health Decision Science, Harvard School of Public Health, 718 Huntington Ave., Boston, MA (United States of America)

Background / Objectives

CAPE (Cervical Cancer Prevention in El Salvador) introduces a low-cost HPV-DNA test into a public sector program. At 19%, El Salvador has one of the lowest screening rates in Latin America. Coverage rates are poor and follow-up for abnormal cytology is inadequate. Started in October 2012, CAPE consists of three phases. The aim is to implement a phased program that will ultimately screen 30,000 women. The true impact of this program lies in its final Phase wherein the program is handed over to the government of El Salvador, and the Ministry of Health makes it the national screening program. Results of phase 2 of the program (n=8,050) are presented.

Methods

8,050 women, age 30-49, were screened in phase 2. 6,737 had both self- and provider-collected careHPV samples and 1,298 had only provider-testing. The agreement between both forms of sampling was 83.6% with a kappa of 0.71. Women with provider-collected HPV-positive results were referred to treatment using the strategy their community followed. Cohort A was referred to colposcopy, and Cohort B had immediate visual triage and was treated with cryotherapy.

Results

Overall, 489 (12.3%) of 3,963 women in Cohort A and 465 (11.4%) of 4,087 women in Cohort B tested HPV-positive. In Cohort A, all were referred for colposcopy—387 (79.1%) attended colposcopy in less than 6 months, and 203/489 (41.5%) were eventually treated. In Cohort B, 397/465 (85%) received immediate treatment and 56 (12%) were referred to colposcopy, of these women 408/465 (87.0%) were eventually treated.

Conclusion

A pilot program introducing HPV testing was successfully implemented in a low-resource setting. Requiring women to return for a colposcopy made them less likely to complete treatment. Outreach to women who had not been screened recently helped find women at higher risk for HPV.

OC 10-01

THE BD ONCLARITY™ HPV ASSAY ON SUREPATH COLLECTED SAMPLES MEETS THE INTERNATIONAL GUIDELINES FOR HUMAN PAPILLOMAVIRUS TEST REQUIREMENTS FOR CERVICAL SCREENING.

D.M. Ejegod¹, F. Bottari², H. Pedersen¹, M.T. Sandri², J. Bonde³

¹1. Department of Pathology, Copenhagen University Hospital, Hvidovre (Denmark), ²2. Division of Laboratory Medicine, European institute of Oncology, Milano (Italy), ³3. Department of Pathology, Copenhagen University Hospital, Hvidovre, Denmark, 3. Clinical Research Centre, Copenhagen University Hospital, Hvidovre (Denmark)

Background / Objectives

To facilitate the highest quality of HPV testing in primary cervical screening, new HPV tests must be evaluated using well annotated samples and in concordance with the International Guidelines for Human Papillomavirus Test Requirements for Cervical Screening also commonly known as the “Meijer Guidelines”. The BD Onclarity HPV assay is the first commercially developed HPV assay to use extended genotyping by single detection of six genotypes (16, 18, 31, 45, 51, 52) and three combined groups of genotypes (33/58, 56/59/66, 35/39/68). Moreover, the assay target the E6 and E7 genes of the HPV virus at DNA level and is therefore not subject to the restrictions of classical L1 assays, or of E6/E7 mRNA assays.

Methods

To assess the performance of the Onclarity assay against the Meijer guidelines we used SurePath collected screening samples from women ≥ 30 years of age participating in the Danish cervical cancer screening program (Ethical committee protocol: H4-2012-070, ClinicalTrials.Gov ID: NCT01671462).

The clinical sensitivity and specificity and assay reproducibility were assessed to validate the Onclarity assay according to the International guidelines. For sensitivity analysis, 61 samples with confirmed \geq CIN2 histology diagnosis were used. For specificity analysis, 1154 samples from women undergoing primary screening without \geq CIN2 diagnosis were used. The intra- and inter-laboratory reproducibility was assessed using 500 samples with known HC2 result. The inter-laboratory reproducibility was performed at the European institute of Oncology, Milan, Italy.

Results

The sensitivity of the Onclarity assay was 96.7% (relative sensitivity compared to HC2 = 0.98) and the specificity was 89.6% (relative specificity compared to HC2 = 1.0). The Onclarity assay was shown to be non-inferior to that of the HC2 assay for both clinical sensitivity (T-test 2.0, P-value=0.02) and specificity (T-test=2.16, P-value=0.02). The Kappa value of the intra-laboratory reproducibility was 0.94 (Lower confidence bound for agreement = 96%) and for inter-laboratory agreement 0.92 (lower confidence bound for agreement = 95%), also meeting the International guidelines.

Conclusion

This is the first study using SurePath samples for clinical validation of any HPV assay. Comparing to HC2, the Onclarity assay obtained validation in concordance with the International guidelines, as also previously reported for the Onclarity assay using ThinPrep samples. Combined with the assays capacity for extended genotyping, the Onclarity assay is a good candidate for use in Danish cervical cancer screening where SurePath is used as collection media.

OC 10-02

COMPARISON OF BD ONCLARITY HPV ASSAY TO ROCHE COBAS 4800 HPV TESTS IN CERVICAL SCREENING IN ENGLAND

K. Ellis, C. Teather, J.H. Smith

Cytology Sheffield Teaching Hospitals NHS FT Sheffield (United kingdom)

Background / Objectives

Human papillomavirus (HPV) testing is routinely used within the National Health Service cervical screening programme (NHSCSP) to triage women with low-grade cytological abnormalities for appropriate referral for colposcopy. The NHSCSP also uses HPV testing for women who have been previously treated for cervical disease known as the 'test of cure' (ToC) and if their negative/low-grade cytology test is HPV negative they are returned to routine surveillance. As new technologies for HPV testing have become available, they have been validated against the Qiagen HC2 assay for approval within the NHSCSP. Thereafter, NHSCSP approved assays can be used for validation of any new assays for introduction within the NHSCSP and a number of these approved tests also offer genotyping at a minimum for high risk HPV types 16, 18 and others. The Roche cobas HPV test uses the polymerase chain reaction (PCR) and real time PCR amplification to detect HPV 16, 18 and 12 other HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The BD Onclarity HPV test is a real time PCR assay too which identifies HPV 16, 18, 45, 31, 51 and 52 individually and groups the other HPV types into three groups (33,58), (56,59,66) and (35,39,68). Here, we compare the performance of the BD Onclarity HPV assay to the Roche cobas 4800 HPV test for overall HPV positivity and for genotyping of HPV 16, 18 and others.

Methods

Cervical samples from women with low-grade cytology and ToC were HPV tested with the BD Onclarity HPV test on the BD Viper LT and the Roche cobas 4800 platform. The BD Onclarity assay results were compared to the Roche cobas 4800 assay and their relative sensitivity assessed. Any women with positive HPV results from either platform were referred for colposcopic assessment.

Results

To date we have compared the HPV results of 1164 cervical samples with low-grade cytology in the triage arm and 673 in the ToC arm. There was 97% agreement for

the two assays in the triage arm and 91.8% in the ToC arm. Both tests so far have detected all CIN2+ cases. Discordant sample results and genotyping comparisons will be presented and discussed.

	cobas POS	Cobas NEG	Grand Total
<i>Triage</i>	872 (74.9%)	292 (25.9%)	1164
Onclarity POS	842 (72.3%)	5 (0.4%)	847
Onclarity NEG	30 (2.6%)	287 (24.7%)	317
<i>ToC</i>	174 (25.9%)	499 (74.1%)	673
Onclarity POS	124 (18.4%)	5 (0.7%)	129
Onclarity NEG	50 (7.4%)	494 (73.4%)	544
Grand Total	1046	791	1837

Conclusion

With the results collated to date, the BD Onclarity HPV assay performed very similarly to the Roche cobas 4800 assay and there was broad agreement on the genotyping.

OC 10-03

HEAD-TO-HEAD COMPARISON OF THE ABBOTT REALTIME HIGH RISK HPV TEST AND THE ROCHE COBAS 4800 HPV TEST IN POPULATION-BASED CERVICAL CANCER SCREENING SETTING

A. Oštrbenk ¹, A. Šterbenc ¹, K. Seme ¹, A. Bertram ², A. Gröning ², M. Poljak ¹

¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana (Slovenia), ²Medizinisches Versorgungszentrum wagnerstibbe für Laboratoriumsmedizin und Pathologie GmbH, Hannover (Germany)

Background / Objectives

The Abbott RealTime High Risk HPV test (RealTime) and Roche cobas 4800 HPV test (cobas) are widely used HPV tests which enable concurrent partial genotyping for HPV16 and HPV18 and aggregate detection of 12 other hr-HPV types. Whereas both tests are considered as clinically validated for primary cervical cancer screening, studies directly comparing their clinical performance are scarce. Objectives: To compare the clinical performance of the RealTime and cobas in a cohort of Slovenian women, who attended the routine organized national cervical cancer screening programme with +70% coverage.

Methods

During the 2009/2010 period, the clinical performance of the RealTime was evaluated on a total of 4,432 women aged 20-64 years. ThinPrep specimens were aliquoted, stored at -80°C and additional testing with cobas was performed in 2015. The main outcome measures were clinical sensitivity for the detection of CIN2+, clinical specificity for the detection of lesions less than CIN2 and predictive values, assessed separately for women >30 years old and for the total study population. Comparisons between both assays were conducted by McNemar's test for matched pairs.

Results

There was a high concordance between the RealTime and cobas, with a kappa value of 0.91 (95% CI, 0.88-0.94) in women >30 years old and 0.92 (95% CI, 0.90-0.94) in total study population. In women >30 years old (n=3,117), the clinical sensitivity and specificity of RealTime were 100.0% (95% CI, 86.5-100.0%) and 93.2% (95% CI,

92.3-94.1%), respectively, and those of cobas were 97.4% (95% CI, 86.2-100.0%) and 92.6% (95% CI, 91.6-93.5%), respectively. In the total study population (n=4,416), the clinical sensitivity and specificity of RealTime were 98.2% (95% CI, 90.6-100.0%) and 89.5% (95% CI, 88.5-90.4%), and those of cobas were 94.7% (95% CI, 85.4-98.9%) and 88.6% (95% CI, 87.6-89.5%), respectively. Although the clinical sensitivity of both tests was comparable in both study groups (p=1.00 and p=0.48), the RealTime was significantly more specific than cobas in women >30 years old (p=0.005) and in total study population (p<0.001). Positive and negative predictive values of RealTime in women >30 years old were 15.4% (95% CI, 11.1-20.5%) and 100.0% (95% CI, 99.8-100.0%), respectively, and of cobas 14.0% (95% CI, 10.1-18.8%) and 100.0% (95% CI, 99.8-100.0%), respectively.

Conclusion

A well balanced clinical performance of high-risk HPV tests has become crucial since the implementation of HPV testing in organized cervical cancer screening programmes. Both RealTime and cobas have high and comparable clinical sensitivity, however, significantly higher clinical specificity of RealTime was obtained in our cohort.

OC 10-04

Triage of women with Low-grade squamous intraepithelial lesion (LSIL) by detection of Human Papillomavirus transformed clonal populations

C. Depuydt¹, E. Somers², M. Dirix², H. Saliha², J. Beert¹, G. Salembier¹, J.P. Bogers¹, W. Tjalma¹

¹Department of Clinical and Molecular Pathology, AML, Sonic Healthcare, Antwerp, Belgium (Belgium), ²Antwerp University Hospital, Centre of Oncologic Research (CORE) Antwerp University, Edegem, Belgium (Belgium)

Background / Objectives

Cervical cytology showing low grade squamous intra-epithelial lesions (L-SIL) is the hallmark of human Papillomavirus (HPV) virion production. When the terminally differentiated non-dividing squamous cells from the cervix are ready to desquamate a new horde of newly formed virions is ready to be released. Most triage algorithms for women with L-SIL is based on detection of high-risk HPV combined with risk calculation to detect high-grade cervical intraepithelial neoplasia (CIN) and cancer (CIN3+). Although some try partial genotyping and only send HPV 16 and or HPV 18 positive women to colposcopy, the majority of these women do not have CIN3+. Moreover no solution is given for women positive for non-HPV 16/18 types. We recently showed that serial viral load measurement allows triage of HPV positive women in transient virion producing infections and HPV transformed clonal populations that can lead to CIN3+. Because these two pathways can occur simultaneously a new algorithm identifying clonal progressing populations was used to triage women with L-SIL.

Methods

Retrospective study using the RIATOL cervical cancer screening and diagnostic follow up database. Since 06/2006, all cervical samples were tested for presence of HPV DNA (18 different quantitative PCRs), before performing cytology. Using the database, we selected women who had a L-SIL smear result in June 2009, had ≥ 2 viral load measurements, and had a subsequent histological result. Changes in HPV specific load between measurements were assessed by linear regression, with calculation of coefficient of determination (R^2) and slope. All detected HPV infections were classified into one of five categories: 1) clonal progressing process ($R^2 \geq 0,85$; positive slope), 2) simultaneously occurring clonal progressive and transient infection, 3) clonal regressing process ($R^2 \geq 0,85$; negative slope), 4) serial transient infection with latency ($R^2 < 0,85$; slopes (2 points) between 0,0010 and -

0,0010 HPV copies/cell/day) and 5) transient productive infection ($R^2 < 0,85$; slope $\pm 0,0099$ HPV copies/cell/day).

Results

260 women with L-SIL were included. Histology results showed that 7.3% developed CIN3, 8.5% CIN2, 27.3% CIN1 and in 41.2% of women the HPV infection regressed. Single HPV infections were detected in 55.8% and multiple HPV infections in 44.2%. In women who developed CIN3, 14 women had multiple infections (73.7%) and only 5 women had single infections (26.3%). In all women with CIN3 a clonal progressive infection could be detected for one of the present HPV types.

Conclusion

Serial type specific viral load measurement detects the HPV type with the clonal progressing viral-load course responsible for CIN3+ in women with LSIL cytology.

OC 10-05

HPV 18 DETECTION VARIABILITY BETWEEN APTIMA® HPV 16 18/45 GENOTYPE AND THE cobas® HPV ASSAY

S.S. Begaj¹, D. Simmons¹, E. Baker²

¹Pathology, Inc. (United States of America), ²Roche Molecular Diagnostics
(United States of America)

Background / Objectives

Human papillomavirus (HPV) is one of the most commonly sexually transmitted infectious pathogens causing cervical cancer in women. Testing for HPV infection is a standard of care as a reflex for ASCUS cytology results in women 21 and older and as a primary adjunctive screen with a Pap test in women 30 to 65 years of age. Genotyping for HPV 16 and 18 genotypes has become a decision making for colposcopy for patients with normal cytology.

The purpose of this study was to determine the HPV18 positivity rate of the APTIMA® HPV 16 18/45 Genotype Assay by testing specimens which are known to be HPV18 positive by **cobas**® 4800 HPV assay in patients with negative for intraepithelial lesion or malignancy (NILM) population. Patient with abnormal Pap results were also included in this study. An additional purpose was to evaluate agreement between the Aptima and the Aptima GT Assays in patients known to be HPV18 positive.

Methods

A total of 279 de-identified residual PreservCyt specimens with previous HPV 18 positive results by the **cobas** test were tested with the Aptima GT Assay. Additionally, 245 of these samples were also tested with the Aptima assay. These specimens included cases with normal and abnormal cytology findings.

Results

The following discrepancies were found between the two assays: 10 cases that were Aptima 18/45 positive were negative for pooled Aptima HPV. 79 cases were HPV 18 positive on **cobas** but HPV 18/45 negative on Aptima; 32 of these had abnormal cytology and 47 had normal cytology. The agreement between the two assays for cases with normal and abnormal cytology was 65.9% and 77.3% respectively, giving

an overall agreement of 71.7%. In comparison to Roche, Aptima had a false negative rate of 17.9%, evenly distributed between cases with normal and abnormal cytology.

Conclusion

Of 279 cases that were HPV 18 positive by **cobas**, the Aptima GT Assay detected 200. Discrepancies were present in 32 cases with abnormal and 47 with normal cytology. Among concordant cases (those detected by both **cobas** and the Aptima GT Assay, there were 10 cases were not detected by the first-line Aptima assay. These cases would not typically be tested with the Aptima GT Assay because the first-line Aptima assay results. This can have significant consequences in patient diagnosis and management.

OC 10-06

PERFORMANCE OF HPV-E7 ONCOPROTEIN
DETECTION AS A TRIAGE METHOD TO COLPOSCOPY
FOR HPV 16/18 POSITIVE WOMEN, COMPARED TO NO
TRIAGE, OR FOR HIGH-RISK HPV (NON16/18)
POSITIVE WOMEN, COMPARED TO CYTOLOGY.
RESULTS OF THE PIPAVIR STUDY.

K. Chatzistamatiou¹, T. Agorastos¹, T. Moysiadis², E. Angelis³, I. Lekka⁴, A. Kaufmann⁵, E. Boschetti-Grützmacher⁵, A. Krings⁵, I. Hagemann⁶, I. Koch⁷, O. Boecher⁷, E. Soutschek⁷, A. Skenderi⁸, S. Angelidou⁹, E. Katsiki⁹, N. Maglaveras⁴, A. Schneider⁵, P. Jansen-Duerr¹⁰

¹4th Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Hippokratio General Hospital, Thessaloniki (Greece), ²Institute of Applied Biosciences, Centre for Research & Technology - Hellas, 57001 Thessaloniki (Greece), ³School of Informatics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki (Greece), ⁴Laboratory of Bioinformatics, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki (Greece), ⁵Department of Gynecology, Charité Campus Benjamin Franklin and Campus Mitte, Berlin (Germany), ⁶MVZ Im Mare. Kiel (Germany), ⁷Mikrogen GmbH, Neuried (Germany), ⁸Laboratory of Cytology, Hippokratio General Hospital, Thessaloniki (Greece), ⁹Department of Histopathology, Hippokratio General Hospital, Thessaloniki (Greece), ¹⁰Research Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck (Austria)

Background / Objectives

High-risk (hr) HPV detection with HPV16/18 genotyping is considered a better method of primary cervical screening than cytology¹⁻⁴, which tends to become a triage test for hr (non16/18) HPV positive women to colposcopy⁵. The objective of the presented study is to assess the performance of the detection of E7HPV protein as a triage method for women positive for either hrHPV (non16/18) or HPV16/18.

Methods

Between February 2013 and April 2014, 1,258 women aged 30-60 years (mean: 43.7), were recruited, at the Family Planning Centre, Hippokratio Hospital, Thessaloniki, Greece, and at the Department of Gynecology, Charité Campus Benjamin Franklin and Campus Mitte, Berlin, Germany, and provided a

cervicovaginal sample, according to the “PIPAVIR” study protocol, which aimed to assess the diagnostic accuracy of HPV DNA test, cytology and E7 test for cervical screening. Cytological evaluation was performed using Liquid Based Cytology (ThinPrep® Hologic, Bedford, MA, USA). An aliquot of each sample was used for hrHPV and E7 detection using HPV Multiplex Genotyping (MPG) and a hrHPV E7 Sandwich ELISA method, developed during the project, respectively. Different hrE7 ELISA formats were used detecting HPV 16/18/31/33/35/39/45/51/52/56/58/59 (recomWell HPV HR screen); HPV16/31/33/35/52/58 (recomWell HPV plus); or HPV16/18/45 (recomWell HPV 16/18/45). Women positive for cytology [atypical squamous cells of undetermined significance or worse (ASCUS+)], hrHPV DNA or E7 were referred for colposcopy. Biopsies and histological assessment of the samples were performed in cases of abnormal colposcopic impression.

Results

Among 1,254 valid tests, hrHPV and HPV16/18 prevalence was 21.7% and 14.7% respectively. Cytology report was ASCUS+ in 7.1%. Cervical Intraepithelial Neoplasia grade 2 or worse (CIN2+) was detected in 25 women (2.0%). For HPV 16/18 positive women with no triage, sensitivity, positive predictive value (PPV) and the number of colposcopies needed to detect one case of CIN2+ were 100.0%, 8.7% and 11.5 respectively. The respective values for E7 testing as a triage method to colposcopy for these women were 100.0%, 15.2% and 6.6. Sensitivity and PPV for the triage of women positive for hrHPV (non 16/18) were 55.5% and 29.4% for cytology; for E7 test the respective values were 77.7% and 24.1%.

Conclusion

Triage, of HPV 16/18 positive women, to colposcopy with the E7 test presents better performance than no triage, decreasing the number of colposcopies needed to detect one CIN2+. In addition, triage of hr (non16/18) HPV positive women with E7 test presents better sensitivity and slightly worse PPV than cytology.

References

- [1] N.W. Bulkmand, L. Rozendaal, P.J. Snijders, F.J. Voorhorst, A.J. Boeke, G.R. Zandwijken, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int J Cancer* 2004 May 20;110(1):94-101.
- [2] P.E. Castle, M.H. Stoler, T.C. Wright, Jr., A. Sharma, T.L. Wright, C.M. Behrens. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *The Lancet Oncology* 2011 Sep;12(9):880-90.

- [3] T. Agorastos, K. Chatzistamatiou, T. Katsamagkas, G. Koliopoulos, A. Daponte, T. Constantinidis, et al. Primary Screening for Cervical Cancer Based on High-Risk Human Papillomavirus (HPV) Detection and HPV 16 and HPV 18 Genotyping, in Comparison to Cytology. PLoS One 2015;10(3):e0119755.
- [4] G. Ronco, J. Dillner, K.M. Elfstrom, S. Tunesi, P.J. Snijders, M. Arbyn, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014 Feb 8;383(9916):524-32.
- [5] W.K. Huh, K.A. Ault, D. Chelmow, D.D. Davey, R.A. Goulart, F.A. Garcia, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Obstetrics and gynecology 2015 Feb;125(2):330-7.

OC 11-01

CLINICAL PERFORMANCE OF GYNTECT - METHYLATION MARKERS FOR TRIAGING HPV- POSITIVE WOMEN

M. Schmitz¹, **A. Hansel**¹, **K. Wunsch**¹, **C. Greinke**², **C. Scheungraber**²,
I.B. Runnebaum², **M. Dürst**²

¹oncgnostics GmbH Jena (Germany), ²Department of Gynecology and
Obstetrics, University Hospital Jena (Germany)

Background / Objectives

High-risk human papillomavirus (hrHPV)-DNA testing is frequently performed after an abnormal cytology result for the detection of high-grade dysplasia and cervical cancer (termed CIN3+), particularly in women above 30 years of age. Although highly sensitive, hrHPV testing has only limited specificity. Therefore, triaging of women tested HPV-positive is of high relevance, even in case of underlying abnormal cytology. Epigenetic markers based on methylation of specific DNA regions during carcinogenesis may have the potential to provide diagnostic tests for triage.

Methods

In a retrospective, cross-sectional study with two gynecological hospitals residual material from cervical scrapes from more than 300 patients with histopathology-confirmed diagnosis were tested with GynTect. For this purpose cellular material was bisulfite-treated, and the GynTect assay using six methylation markers and two internal controls for detecting women with relevant CIN3+ lesions, was performed.

Results

All cancer cases and 66% of CIN3 were detected with the GynTect assay. Among women older than 29 years of age, almost 80% of the CIN3 cases were detected. In the no CIN group, several cases were detected that showed a high methylation grade and a positive p16 result in the corresponding biopsy, suggesting that GynTect may detect an underlying dysplasia very early. Therefore, such cases require clarification. In total, sensitivity and specificity in this cohort for CIN3+ was 68% and 83%, respectively.

Conclusion

GynTect provides a promising diagnostic tool for identifying patients with CIN3+ among hrHPV-positively tested women. A follow-up of all patients having high methylation level in the no CIN group should show that methylation of the GynTect markers is a strong indication for an underlying CIN that requires close monitoring of the corresponding patients.

OC 11-02

GENOME-WIDE METHYLOME ANALYSIS UNCOVERS NEW HYPERMETHYLATION BIOMARKERS FOR BOTH ADENO- AND SQUAMOUS CELL CERVICAL CARCINOMA.

R. Van Leeuwen¹, R. Wang², A. Boers¹, T. De Meyer³, R. Steenbergen⁴, W. Van Criekinge³, A. Van Der Zee¹, E. Schuurin⁵, B. Wisman¹

¹Department of Gynaecologic Oncology, Cancer Research Centre Groningen, University of Groningen, University Medical Centre Groningen, Groningen (Netherlands), ²Department of Laboratory Medicine, Tianjin Medical University, Tianjin (China), ³Department of Mathematical Modelling, Statistics and Bioinformatics, University of Ghent, Ghent (Belgium), ⁴Department of Pathology, VU University Medical Centre, Amsterdam (Netherlands), ⁵Department of Pathology, Cancer Research Centre Groningen, University of Groningen, University Medical Centre Groningen, Groningen (Netherlands)

Background / Objectives

The sensitivity of cytology-based screening methods for adenocarcinoma of the cervix (ADC) and its precursors is low compared to the already suboptimal sensitivity for squamous-cell cervical neoplasia for a variety of reasons. Analysis of DNA methylation markers has been reported promising to detect squamous-cell carcinoma (SCC) in earlier stages, but few methylation markers have been described for the early detection of ADC. The aim of this study was to identify methylation markers for the early detection of both ADC and SCC.

Methods

Global methylation profiles were generated for frozen tissues of 20 normal cervixes, 6 ADC and 6 SCC by capturing methylated DNA and performing next-generation sequencing (MethylCap-seq). Differentially methylated regions were appraised by means of a step-wise validation approach using bisulfite pyrosequencing and methylation-specific PCR (MSP) on various cohorts of patient material (i.e. formalin-fixed paraffin-embedded (FFPE) tissues of 17 normal cervixes and 6 ADC and 7 SCC; 225 cervical scrapings from women either with or without cervical cancer; and 229 scrapings of women who were referred for colposcopy).

Results

MethylCap-seq revealed 53 regions that were methylated in both ADC and SCC and not in normal cervixes. Of the 15 most significant regions, 5 markers exhibited significantly different methylation rates between normal and cancer FFPE tissues. Quantitative MSP (QMSP) on cervical scrapings from an independent cohort of 89 women with a normal cervix and 68 patients with SCC and 57 with ADC, revealed a sensitivity of 79% to 88% to detect cervical cancer, while 94% to 99% of normal scrapings tested negative. The QMSPs for 4 markers (SOX1, SOX14, SLC6A5 and TBX20) detected ADC and SCC with a similar sensitivity. Finally, the analysis of scrapings from a cohort of women referred with an abnormal smear with known histological diagnosis (n=229 including 14 adenocarcinoma in situ (AdCIS), 12 ADC and 29 SCC), revealed that the individual QMSPs were able to detect cervical intraepithelial neoplasia grade 2 or worse (CIN2+) with a sensitivity ranging from 28% to 59%, of which the AdCIS showed a sensitivity of 71-93%, and with a high specificity (88-98%). Compared to hrHPV testing, the combination of SOX1 or SOX14 methylation showed a similar sensitivity for CIN3+ (73% vs. 80% for hrHPV, $p > .2$), whereas the specificity was significantly better (88% vs. 42% for hrHPV, $p < 10^{-5}$).

Conclusion

We identified 4 new methylation markers with a high sensitivity for both ADC as well as SCC in cervical scrapings. Additionally, the combined methylation of SOX1 or SOX14 showed a better specificity compared to HPV analysis.

OC 11-03

PERFORMANCE OF CADM1/MAL-METHYLATION ANALYSIS FOR MONITORING WOMEN TREATED FOR HIGH-GRADE CIN

M. Van Zummeren¹, M.H. Uijterwaal¹, M. Kocken¹, R. Luttmmer¹, J. Berkhof², B.I. Witte², M.W. Van Baal³, P.G.C.M. Graziosi⁴, R.H.M. Verheijen⁵, T.J.M. Helmerhorst⁶, D.K.E. Van Dijken⁷, J.W. Spruijt⁸, F.J. Van Kemenade⁹, D.A.M. Heideman¹, P.J.F. Snijders¹, R.D.M. Steenbergen¹, C.J.L.M. Meijer¹

¹Department of Pathology, VU University Medical Center, Amsterdam (Netherlands), ²Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands (Netherlands), ³Department of Obstetrics and Gynecology, Flevo Hospital, Almere, The Netherlands (Netherlands), ⁴Department of Obstetrics and Gynecology, Sint Antonius Hospital, Nieuwegein, The Netherlands (Netherlands), ⁵Department of Women and Baby, University Medical Center Utrecht, The Netherlands (Netherlands), ⁶Department of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, The Netherlands (Netherlands), ⁷Department of Obstetrics and Gynecology, Onze Lieve Vrouwen Gasthuis West, Amsterdam, The Netherlands (Netherlands), ⁸Department of Obstetrics and Gynecology, VU University Medical Center, Amsterdam, The Netherlands (Netherlands), ⁹Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands (Netherlands)

Background / Objectives

Recent studies have shown that a negative CADM1/MAL-methylation test reassures against cervical cancer and detects high-grade CIN lesions with a high short-term risk of progression to cervical cancer. Women treated for CIN2/3 are at risk of post-treatment disease, representing either persistent (incompletely treated) or incident (newly developed) lesions. Here, we evaluated CADM1/MAL-methylation analysis as potential tool for detecting recurrent high-grade CIN lesions (rCIN2/3).

Methods

A multicenter prospective clinical cohort study was conducted among 364 women treated for CIN2/3. Cervical scrapes were taken prior to treatment, and 6 and 12 months post-treatment and tested for cytology, hrHPV (plus genotype) and CADM1/MAL-methylation. If at 6 months either of these tests was positive, a colposcopy-directed biopsy was obtained. At 12 months, all women underwent an

exit-colposcopy with mandatory biopsy. In case of rCIN2/3, a re-treatment (re-LLETZ) was done. Primary outcome measure was \geq rCIN2 at 6 or 12 months post-treatment.

Results

We found 28 rCIN2 (7.7%) and 14 rCIN3 (3.8%), resulting in a total recurrence percentage of 11.5%. All 14 women with rCIN3 and 15/28 (54%) with rCIN2 showed hrHPV type-persistence. Of these, 9/14 (64%) rCIN3 and 8/15 (53%) rCIN2 were CADM1/MAL-methylation positive. The 5 incident (hrHPV type-switch) rCIN2 were all CADM1/MAL-methylation negative, whereas the 3 carcinomas found after re-treatment were all CADM1/MAL-methylation positive. CADM1/MAL-methylation positivity at baseline and in follow-up significantly increased the risk of \geq rCIN3 from 0.7% to 18.4% respectively, and for \geq rCIN2 from 8.2% to 36.8%, compared to negative test results (p-value: <0.001).

Conclusion

Post-treatment monitoring by CADM1/MAL-methylation analysis identifies women with an increased risk of rCIN2/3. Moreover, our results confirm previous data indicating that CADM1/MAL-methylation analysis provides a high reassurance against cancer.

OC 11-04

EFFECTS OF HPV 16 E6 AND E7 ON GENOMIC STABILITY IN HCT116 CELLS

L. Ganss¹, S. Vinokurova², S. Duensing³, M. Von Knebel-Doeberitz¹

¹Department of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, and Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), 69120 Heidelberg (Germany), ²N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Moscow 115478 (Russian federation), ³Molecular Urooncology, Department of Urology, University of Heidelberg School of Medicine (Germany)

Background / Objectives

Genomic instability develops at early stages of HPV-infected neoplasias and is associated with deregulated expression of the oncogenes E6 and E7, which were both shown to induce centrosome abnormalities, multipolar mitosis and aneuploidy [1, 2]. The effects of HPV 16 E6 and E7 on genomic integrity have been described in primary keratinocytes and in cervical cancer cell lines, which are either critical for long-term culturing or already chromosomally unstable. To analyze the effects of the HPV oncogenes on genomic stability in a time dependent manner we intended to use chromosomally stable HCT116 colon carcinoma cells for the generation of clones that allow doxycycline inducible expression of HPV 16 E6 and E7.

Methods

Western Blotting and RT-qPCR were performed to characterize HPV 16 E6 and E7 expression in selected doxycycline inducible HCT116 clones. Effects on centrosome numbers and spindle pole formation during mitosis were analyzed using γ -tubulin immunostainings. DNA damage in HCT116 clones induced for E6 and E7 expression was evaluated by staining of the phosphorylated histone component γ H2AX, a marker for DNA double strand breaks. The induction of DNA single and double strand breaks were further analyzed by performing Comet Assay.

Results

Induction of both oncogenes elevated the number of interphase cells showing abnormal centrosome numbers. Additionally, the percentage of cells containing abnormal spindle poles during mitosis was significantly increased. Both effects could already be observed after 48 hours of oncogene induction and were found to be elevated after longer induction phases. Induction of E6 and E7 expression also affected DNA integrity of HCT116 cells. Moderate increases in γ H2AX

phosphorylation indicating DNA double strand break formation could be observed in response to E6 and E7 expression.

Conclusion

The results of the present study suggest that expression of both HPV 16 oncogenes affects the genomic stability in HCT116 cells. Induction of abnormal centrosome numbers may result in aberrant spindle pole formation during mitosis increasing the number of DNA damaged daughter cells. Despite the fact that severely DNA damaged cells potentially undergo apoptosis, the effects of E6 and E7 on mitotic progression might increase the genomic variability promoting the outgrowth of selected cells. As these effects could already be observed 48 hours after inducing the expression of the HPV 16 oncogenes, they may represent a very early event during HPV-mediated transformation.

References

1. Duensing S, Lee LY, Duensing A, Basile J, Piboonniyom S, Gonzalez S, Crum CP and Munger K. The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proc Natl Acad Sci U S A*. 2000; 97(18):10002-10007.
2. Duensing S and Munger K. The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability. *Cancer Research*. 2002; 62(23):7075-7082.

OC 11-05

P16INK4A IMMUNOHISTOCHEMISTRY / HPV DNA PCR CO-TESTING IDENTIFIES HPV-INDUCED ANAL SQUAMOUS CELL CARCINOMAS WITH HIGH DIAGNOSTIC ACCURACY

T. Obermueller¹, M. Reuschenbach¹, S.A. Koerber², J. Debus², F. Sterzing², M. Von Knebel Doeberitz¹, E.S. Prigge¹

¹Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany (Germany), ²Department of Radiation Oncology, University Hospital Heidelberg, Heidelberg, Germany (Germany)

Background / Objectives

Squamous cell carcinomas represent the most common histologic entity of all anal cancers (ASCC). Human papillomavirus (HPV) DNA has been detected in up to 90% of ASCC in previous studies. The frequent accompanying overexpression of the cell cycle protein p16^{INK4A} has indicated an etiological association with HPV in a large proportion of ASCC. However, the gold standard to prove etiological relevance of HPV in carcinogenesis is the detection of HPV E6/E7 oncogene transcripts. The aim of this study was to identify the proportion of ASCC that are etiologically driven by HPV in a German cohort of 82 ASCC. Furthermore, the diagnostic value of HPV DNA detection and p16^{INK4A} immunohistochemistry (IHC) alone and in combination to indicate HPV-induced ASCC was analyzed.

Methods

82 patients diagnosed with ASCC at the University Hospital Heidelberg, Germany between 2000 and 2011 were included in the study. Previously, the samples had been tested for HPV DNA using Luminex technology and p16^{INK4A} immunohistochemical expression. ASCC containing $\geq 50\%$ p16^{INK4A}-overexpressing tumor cells were considered p16^{INK4A}-positive. A transforming relevance of HPV in the ASCC was analyzed by E6*I mRNA detection using quantitative real-time-PCR. E6*I mRNA is a splice variant product of the E6 full-length transcript which is generally highly expressed in HPV-transformed cells. Sensitivity and specificity of HPV DNA PCR and p16^{INK4A} IHC alone or in combination to detect a transforming HPV-infection (using E6*I identification as gold standard) were analyzed.

Results

Eighty-four percent (69/82) of the samples were tested positive for HPV 16/18 DNA. Based on IHC, 82% (67/82) of the ASCC demonstrated overexpression of p16^{INK4A}. E6*I mRNA was identified in 79.3% (65/82) of samples. Single application of the surrogate markers p16^{INK4A} IHC or HPV DNA showed high sensitivity (98.5% and 100.0%, respectively) and moderate specificity (82.3% and 76.5%, respectively.). Co-testing for p16^{INK4A} IHC and HPV DNA PCR demonstrated high sensitivity (98.4%) and excellent specificity (100.0%) for a transforming High Risk-HPV-infection (HR-HPV) in ASCC.

Conclusion

About 80% of ASCC of the analyzed cohort were shown to be etiologically driven by HR-HPV. The exclusive detection of HR-HPV DNA or p16^{INK4A} overexpression demonstrates moderate specificity for a transforming HPV-infection. However, the combination of the surrogate markers identifies a transforming HPV-infection in ASCC with a high sensitivity and excellent specificity. In conclusion, co-testing of p16^{INK4A} IHC and HPV DNA PCR is suggested as a reliable test combination to identify HPV-driven ASCC with high diagnostic accuracy.

OC 11-06

Characterization of cervical lesions by expression analysis of p16 and Stathmin

N. Nevermann¹, M. Basten¹, D. Schiller¹, A. Schneider², N. Choly², E. Boschetti-Grützmacher¹, A.M. Kaufmann¹

¹Clinic for Gynecology, Charité-Universitätsmedizin Berlin (Germany), ²MVZ Im Fürstenbergkarree, Berlin, Germany (Germany)

Background / Objectives

The use of biomarkers for characterizing HPV infections and diagnosing cervical cancer precursors has become a central topic in cervical cancer screening. Our study focused on p16 known as a marker for high risk HPV-infection that is expressed in 100% of lesions with CIN2 or higher and on Stathmin (Oncoprotein18, STMN), a marker that might be correlating with higher severity of intracervical lesions. The strength of expression of STMN over p16 might be an indicator for risk of dysplasia within a high-risk HPV infection and therefore serve as a good predictor for the severity of a lesion.

Methods

In a quantitative molecular assay we compared levels of mRNA expression of p16 and stathmin. This method is a multiplex assay combining specific mRNA capture and signal amplification with multi-analyte profiling beads (xMAP) technologies to simultaneously detect and quantify RNA targets. This allows a quantitative analysis. Values are arbitrary mean fluorescence intensity (MFI). P16 and STMN were divided by the MFI value for Actin β as a housekeeping gene and therefore put in relation to the absolute cell number.

A total of 178 cervical smears conserved in Thinprep were analysed. HPV genotyping (with multiplex human papillomavirus genotyping (MPG) and a Luminex read-out) was performed as part of the clinical diagnostics and clinical data for cytology, colposcopy and histology were used for classification of the lesions.

Results

We found a median expression of p16 mRNA of 18.22 for samples with no cervical lesions (NILM, n=52), 6.42 for CIN1 (n=13), 19.03 for CIN2 (n=29), 25.31 for CIN3 (n=69) and 79.08 for invasive carcinomas (n=15).

Median expression of STMN mRNA was 20.74 for samples with no cervical lesions (NILM), 20.33 for CIN1, 19.27 for CIN2, 51.70 for CIN3 and 291.20 for invasive carcinomas.

We found an average ratio for STMN/p16 mRNA expression of 1.64 for patients with no cervical lesion (median: 0.8, standard deviation (SD): 2.03), 2.25 for CIN1 (median: 1.42, SD: 2.15), 1.43 for CIN 2 (median: 0.6, SD: 1.8), 4.61 for CIN3 (median: 1.67, SD: 10.77) and 12.91 for invasive carcinomas (median: 6.89, SD: 17.60).

Conclusion

While p16 is an indicator for high risk HPV infections, STMN shows a stronger increase of expression in CIN3 +. The ratio of STMN/p16 might therefore discriminate low risk of transformation and high grade dysplasia within lesions with hr HPV infection. This information could be of value in HPV diagnostics and can not yet be retrieved from other testing methods.

OC 11-07

P16/Ki-67 dual-stained cytology for detecting cervical (pre)cancer in a HPV-positive gynecologic outpatient population

R. Luttmer¹, **M. Dijkstra**¹, **P. Snijders**¹, **J. Berkhof**¹, **F. Van Kemenade**², **L. Rozendaal**¹, **T. Helmerhorst**², **R. Verheijen**³, **A. Ter Harmsel**⁴, **M. Van Baal**⁵, **P. Graziosi**⁶, **W. Quint**⁷, **J. Spruijt**¹, **D. Van Dijken**⁸, **D. Heideman**¹, **C. Meijer**¹

¹VU University Medical Center Amsterdam (Netherlands), ²Erasmus Medical Center Rotterdam (Netherlands), ³UMC Utrecht Cancer Center (Netherlands), ⁴Roosevelt Clinic Leiden (Netherlands), ⁵Flevo Hospital Almere (Netherlands), ⁶Sint Antonius Hospital Nieuwegein (Netherlands), ⁷DDL Diagnostic Laboratory Rijswijk (Netherlands), ⁸Onze Lieve Vrouwe Gasthuis Amsterdam (Netherlands)

Background / Objectives

High-risk human papillomavirus (hrHPV) positive women require triage testing to identify those with high-grade cervical intraepithelial neoplasia or cancer (\geq CIN3). Although Pap cytology is considered an attractive triage test, its applicability is hampered by its subjective nature. This study prospectively compared the clinical performance of p16/Ki-67 dual stained cytology to that of Pap cytology, with or without HPV16/18 genotyping, in hrHPV-positive women.

Methods

Among women visiting gynaecologic outpatient clinics, 446 high-risk HPV-positive women (age 18-66 years) were recruited. From all women, cervical scrapes and colposcopy-directed biopsies were obtained. Cervical scrapes were subjected to Pap cytology, HPV16/18 genotyping and p16/Ki-67-dual stained cytology.

Results

The \geq CIN3 sensitivity of p16/Ki-67 dual-stained cytology (93.8%) did neither differ significantly from that of Pap cytology (87.7%; ratio 1.07, 95% CI:0.97-1.18) nor from that of Pap cytology combined with HPV16/18 genotyping (95.1%; ratio 0.99, 95% CI:0.91-1.07). However, the \geq CIN3 specificity of p16/Ki-67 dual stained cytology (51.2%) was significantly higher than that of Pap cytology (44.9%; ratio 1.14, 95% CI:1.01-1.29) and Pap cytology combined with HPV16/18 genotyping (25.8%; ratio 1.99, 95% CI:1.68-2.35). After exclusion of women who had been referred because of abnormal Pap cytology, the \geq CIN3 specificity of p16/Ki-67 dual stained cytology

(56.7%) remained the same, whereas that of Pap cytology (60.3%) increased substantially, resulting in a similar specificity of both assays (ratio 0.94, 95% CI:0.83-1.07) in this sub-cohort.

Conclusion

p16/Ki-67 dual-stained cytology may serve as a more objective alternative to Pap cytology for triage of hrHPV-positive women.

OC 11-08

16/Ki-67 AS A TRIAGE TEST IN ROUTINE: CORRELATION WITH HISTOLOGY

A. Xhaja, C. Börsch, I. Zeiser, A. Bernhard, H. Ikenberg

**Cytomol, Laboratory for Cytology and Molecular Biology, D-60437 Frankfurt,
Germany (Germany)**

Background / Objectives

Immunostaining for p16/Ki-67 (CINtec® Plus, Roche Ventana, Mannheim, Germany) is valuable for the triage of borderline and low-grade cytological abnormalities as well as of HPV-high-risk (HR) -positive, cytologically negative or HPV-HR-negative, cytologically positive cases. We analyzed the correlation between the p16/Ki-67 status of patients before biopsy/therapy with histologically confirmed CIN 2 and 3 and invasive cervical carcinoma.

Methods

All cases of a German routine lab in which histology had been performed in 2012 and in 2013 and which had a test of HPV-HR (cobas®, Roche Diagnostics, Mannheim, Germany) and p16/Ki-67, both carried out maximally six months earlier, are reported. Histology followed colposcopically directed biopsy, conization or hysterectomy. p16/Ki-67 tests were made out of cervical smears taken in liquid based cytology medium (PreservCyt®, Hologic, Wiesbaden, Germany) according to the manufactures instructions.

Results

In 2012, in 674 of 1167 CIN 2+ cases (57.8%) and in 2013, in 578 of 1004 CIN 2+ cases (57.6%) a p16/Ki-67 result was available. For both years together in CIN 2 270 (94.73%), in CIN 3 939 (99.8%) and in cervical cancer 26 (100%) cases were p16/Ki-67 positive. In all histologically confirmed CIN 2+ cases we found 11 HPV-HR-negative CIN 2 (2.57%), 23 HPV-HR-negative CIN 3 (1.85%) and 5 HPV-HR-negative (10.5%) invasive cervical cancer cases. In 27 from 39 (69%) HPV-HR-negative cases a p16/Ki-67 cotest was available. 7 of 8 CIN 2 (87.5%), 17 of 18 CIN 3 (95%) and 1 of 1 (100%) cervical cancer HPV-HR-negative cases were p16/Ki-67 positive.

Conclusion

The large majority of histologically confirmed CIN 2 and 3 and cervical cancers were positive for the biomarker p16/Ki-67 when tested in cervical smears \leq 6 months before biopsy/therapy. **In 25 from 27 (93%) HPV-negative cases with p16/Ki-67 cotesting the p16/Ki-76 test was positive. 965 from 967 (99.8%) of all histologically confirmed CIN 3+ lesions tested with p16/Ki67 were positive**

OC 11-09

COMBINED BIOMARKER EXPRESSION PATTERNS OF panHPVE4 AND p16INK4a CAN SUPPORT THE DIAGNOSIS AND GRADING OF CIN

A. Leeman¹, A. Kasius¹, M. Del Pino², A. Rodriguez², A. Torne², J. Ordi³, H. Griffin⁴, M. Van De Sandt¹, A. Molijn¹, F. Van Kemenade⁵, D. Jenkins¹, J. Doorbar⁴, W. Quint¹

¹DDL Diagnostic Laboratory, Visseringlaan 25, 2288 ER Rijswijk (Netherlands), ²Institute of Gynecology, Obstetrics and Neonatology, Hospital Clínic Clínic—Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona (Spain), ³Department of Pathology, Hospital Clínic, Barcelona (Spain), ⁴University of Cambridge, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP (United kingdom), ⁵Erasmus Medical Center, Department of Pathology, PO Box 2040, 3000 CA Rotterdam (Netherlands)

Background / Objectives

In the prevention of cervical cancer, cervical intraepithelial neoplasia grade 2 (CIN2) is currently the treatment threshold. However, reproducibility of CIN2 diagnosis is poor because it consists of a mixture of productive infections with a high chance of regression and transforming infections with a low chance of regression. Biomarkers that can distinguish the two are urgently needed to reduce overtreatment. Expression patterns of panHPV-E4, a marker for human papillomavirus (HPV) life-cycle completion, and p16INK4a, which indicates transforming HPV-related lesion, may contribute.

Objective: To assess the expression patterns of immunohistochemical markers E4 and p16 in different grades of CIN.

Methods

Biopsies (78 negative, 54 CIN1, 65 CIN2, 52 CIN3, 1 adenocarcinoma in situ (AIS), 2 carcinomas) from 252 women were stained with E4 (panHPVE4 mAb FH1.1 detecting at least 16 HR-HPV types) and p16INK4a clone E6H4 (Ventana Medical Systems, Tucson, AZ). The worst lesion in a biopsy was scored by a pathologist for both markers with respect to the expression pattern: negative, extensive positivity or focal positivity for E4 and negative, patchy positivity, positivity in the lower 1/3 of the epithelium, positivity in the lower 2/3 or full thickness for p16.

Results

All histologically negative biopsies were negative for E4. Of the CIN1 lesions, 63% was negative, 30% showed extensive positivity and 7% showed focal positivity. In the CIN2 lesions, these numbers were resp. 65%, 11% and 24%. Of the CIN3 lesions, 98% was negative and 2% showed focal positivity. The AIS and both carcinomas were E4 negative.

With p16 staining, 18% of the histologically negative lesions showed patchy p16 positivity. For CIN1, CIN2 and CIN3 the positivity rates were resp. 83% (incl. 20% patchy), 98% and 96%. AIS and both carcinomas showed full thickness p16 positivity. Patterns of positivity of both biomarkers combined are shown in Table 1.

Table 1: E4 and p16 biomarker staining pattern in different grades of CIN

Diagnosis	E4-/p16-	E4+/p16-	E4+/p16+	E4-/p16+
Negative	83%	0%	0%	18%
CIN1	9%	7%	30%	54%
CIN2	2%	0%	35%	63%
CIN3	4%	0%	2%	94%
AIS	0%	0%	0%	100%
Carcinoma	0%	0%	0%	100%
Total	73	4	40	135

Conclusion

E4 and p16 show distinct staining patterns in different grades of CIN. Histologically negative lesions are E4 negative with limited or no p16 staining. CIN3 lesions are E4 negative with extensive p16 staining. Neither histological CIN1 nor CIN2 are homogeneous groups and include lesions that can be classified as productive, with extensive E4 staining and lower 1/3 p16 staining, or as intermediate, with E4 staining and more extensive p16 staining, or as transforming, with extensive p16 expression. E4 and p16 combined staining could provide a clinically useful, more objective assessment of biopsies.

OC 11-10

HIGH SENSITIVITY PROTEOMIC ANALYSIS REVEALS NOVEL PATHWAYS AND KEY REGULATORS IN THE PATHOLOGY OF CERVICAL CANCER

V. Lygirou¹, G. Kontostathi¹, J. Zoidakis¹, A. Vlahou¹, N.P. Anagnou², K.I. Pappa³

¹Biotechnology Laboratory, Centre of Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece (Greece), ²Cell and Gene Therapy Laboratory, Centre of Basic Research II, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece (Greece), ³First Department of Obstetrics and Gynecology, University of Athens School of Medicine, Athens, Greece (Greece)

Background / Objectives

Cervical cancer is the third most common and fourth most fatal type of cancer among women. Our study aims in the elucidation of the molecular mechanisms underlying malignant transformation of the cervical epithelium. To achieve this goal, we used state-of-the-art proteomics techniques.

Methods

The pattern of the total cell extract of cervical cancer cell lines HeLa (HPV18+), SiHa (HPV16+), and C-33A (HPV-), was compared to normal cervical keratinocytes (HCK1T). The peptides extracted from each sample after in-gel tryptic digestion, were analyzed by Liquid Chromatography coupled to high resolution mass spectrometry (LC-MS/MS). Bioinformatics analysis was performed on the results utilizing various platforms and databases (e.g. Ingenuity Pathway Analysis, Cytoscape). Validation of the in vitro results was performed with Multiple Reaction Monitoring – Mass Spectrometry (MRM-MS), a highly sensitive and selective, targeted proteomics approach for peptide quantitation. Clinical validation was performed with immunohistochemistry.

Results

The proteomics analysis yielded a high number of identifications for each cell line (~2500-3500 proteins), with a satisfying reproducibility among biological replicates. Moreover, the comparison between each cancer cell line and the normal keratinocytes resulted in ~900-1400 statistically significant ($p < 0.05$, Mann Whitney) differences in the proteome. The total number of differentially expressed proteins was

analyzed by bioinformatics tools in order to investigate the biological processes and molecular pathways that contribute to carcinogenesis and metastasis in the cervical epithelium. Two transcription factors, p53 and c-Myc, that are known to regulate cervical carcinogenesis, were predicted to control a significant number of differentially expressed proteins in our dataset. Actin cytoskeleton signaling, which plays an important role in carcinogenesis and metastasis, is a prominent pathway predicted by Ingenuity Pathway Analysis based on the proteomics results. Other pathways and proteins potentially involved in the pathology of cervical cancer identified from this analysis included translation and Eukaryotic Initiation Factor 2 (eIF2). Interesting molecules, that appear to have key roles in cervical cancer, based on this analysis, were chosen and further validated by MRM-MS and Immunohistochemistry in cervical cancer cell lines and clinical samples.

Conclusion

This systems biology approach provided new insights in cervical cancer pathology and led to the discovery of potential biomarkers and pharmacological targets.

OC 11-11

Performance of a New HPV and Biomarker Assay in the Management of High Risk HPV Positive Cases

A. Kocsis¹, **Z. Schaff**², **R. Koiss**³, **B. Járny**⁴, **G. Sobel**², **K. Pap**⁵, **I. Székely**³, **H.C. Lai**⁶, **T. Takács**⁴, **M. Nyíri**¹, **M. Benczik**⁴

¹NEUMANN DX Ltd., Cellcall Ltd. (Hungary), ²2nd Department of Pathology, Semmelweis University (Hungary), ³Department of Obstetrics and Gynaecology, St. Stephan Hospital (Hungary), ⁴NEUMANN DX Ltd., Cellcall Ltd., Synlab Hungary Ltd. (Hungary), ⁵Jósa András Regional Central Hospital (Hungary), ⁶Shunag Ho Hospital, Taipei Medical University (Hungary)

Background / Objectives

Objectives. The most recent guidelines recommend primary hrHPV screening as an alternative to cytology-based cervical cancer screening. Although hrHPV detection has high sensitivity, it is not specific enough, leading to a substantial false positive rate. In our ongoing multicentric clinical study we investigated an epigenetic molecular biomarker, based on host gene methylation, as a potential triage method of hrHPV positive women.

Methods

Methods. In the presented multicentric clinical study over 6,000 women were enrolled to assess the performance of the CONFIDENCE™ assay. Cervical specimens were obtained from women aged between 18 and 65 years, who attend cervical sampling at 4 clinical sites among outpatient and colposcopy clinics in Hungary. LBC cytology, hrHPV detection (CONFIDENCE™ HPV, NEUMANN Ltd.; and cobas® HPV, Roche or Full Spectrum HPV, GenID/Synlab as comparator) and single target (POU4F3) host-gene methylation test (CONFIDENCE™ Marker, NEUMANN Ltd.) were performed using the same LBC sample in all cases independently from the HPV status. In clinically indicated 116 cases, diagnostic histology was used as a 'gold standard' reference method. In the absence of histologically confirmed diagnosis, negative histology result was presumed. The current analysis is focused on the baseline cross-sectional clinical data of 5320 LBC samples above 25 years of age.

Results

Results. Overall 20.46% of the LBC samples was hrHPV positive. CONFIDENCE™ HPV test was compared to cobas HPV on 3270 samples, and to Full Spectrum HPV

in 2280 cases. We found 70.4% and 61.4% agreement in the hrHPV positive cases, with overall kappa value 0.78 (95% CI: 0.74-0.81) and 0.70 (95% CI: 0.66-0.74), which is a very good agreement. The sensitivities of POU4F3 in discriminating CIN2+ and CIN3+ among hrHPV positive women were 90.0% (95% CI: 81.2-95.6%) and 92.4% (95% CI: 83.2-97.5%), whereas the specificities were 75.3% (95% CI: 72.7-77.7%) and 74.7% (95% CI: 72.1-77.1%) respectively. Positive and negative predictive value was 20.1% (95% CI: 16.1-24.6%) and 99.1% (95% CI: 98.2-99.6%) for CIN2+. The methylation of POU4F3 showed increased sensitivity with similar specificity comparing to cytology. The range of sensitivity was equivalent to cytology extended with HPV16/18 genotyping.

Conclusion

Conclusion. Our study provides the largest clinical evaluation of a single host gene methylation biomarker using in hrHPV triage so far. In our subanalysis POU4F3 exceeds the recently described sensitivity and specificity in the field of epigenetic biomarkers of cervical precancer. POU4F3 alone shows comparable or better performance, than other methylation markers alone or in panel.

OC 11-12

TRACING HPV DNA INTEGRATION SITES DURING THE DEVELOPMENT OF PRE-CANCEROUS LESIONS OF THE CERVIX

K. Carow¹, **S. Chotewutmontri**², **U. Klos**³, **C. Scheungraber**¹, **I.B. Runnebaum**¹, **E. Schwarz**³, **M. Dürst**¹

¹Department of Gynaecology, Jena University Hospital – Friedrich Schiller University Jena, 07743 Jena (Germany), ²Genomics and Proteomics Core Facility, DKFZ, Heidelberg (Germany), ³Research Program Infection, Inflammation and Cancer, DKFZ, Heidelberg (Germany)

Background / Objectives

HPV DNA integration into the host genome is a characteristic step in cervical carcinogenesis and seems to contribute to clonal expansion of malignant lesions. Indirect techniques for assessing the physical state of the viral genome such as the determination of E2/E6 ratios suggest that virus integration occurs frequently already in low grade lesions. However, this has not been confirmed at the sequence level. The aim of this study is to identify and sequence the integration sites in CIN3 and to use these sequence data to trace the integration sites in serial cervical swabs taken during the course of disease development. By this approach we will determine the time points in the natural history of CIN lesions at which driver-integrates can be detected first.

Methods

We included cervical swab samples from 22 patients with histologically confirmed HPV16-positive CIN3 who were treated between 2011 and 2015 at Jena University Hospital. Sample material comprised on average 4 cervical swabs per patient, collected at different time points up to 2 years prior to the diagnosis of CIN3. DNA was isolated using the NucleoSpin® Tissue kit from Macherey Nagel. HPV DNA integration sites were identified and sequenced in swabs taken from CIN3 lesions applying our recently published TEN16 approach (1). Validation of integration sites is done via PCR with primers flanking the transition of HPV and human DNA (viral-cellular junction PCR, vcj-PCR). In case of successful integrate validation, vcj-PCR is used to screen swabs taken prior to the diagnosis of CIN3.

Results

So far, we have analysed the integration state in 18 of 22 cases with CIN3. For 4 patients HPV16 DNA integration sites could be identified and validated by vcj-PCR. The latter technique allows the detection of a single integrate in a background of 1000 integrate-free cells. Up to 3 different patient-specific integration sites were detected. The vcj-PCR is now being used to detect the integration sites in swabs collected prior to the diagnosis of CIN3.

Conclusion

We have demonstrated that the identification of integrated HPV16 DNA from cervical swabs of patients with CIN3 is feasible. The integration rate in this cohort is only 22% up to now. Further analysis and optimization of the TEN16 assay may reveal a higher integration rate since integration rates of up to 50 % have been described in other studies. Should we be able to trace driver integration events to early stages of disease the TEN16 platform may become a powerful prognostic tool.

References

B. Xu et al., Multiplex Identification of Human Papillomavirus 16 DNA Integration Sites in Cervical Carcinomas. PLoS One 8, e66693 (2013).

OC 11-13

METABOLOMICS OF CERVICAL CANCER CELL LINES DOCUMENT DISCRETE PROFILES AND REVEAL NOVEL METABOLITES WITH HPV-SPECIFIC FEATURES

K. Pappa¹, N. Anagnostou²

¹Cell and Gene Therapy Laboratory, Centre of Basic Research II, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece and First Department of Obstetrics and Gynecology, University of Athens School (Greece), ²Cell and Gene Therapy Laboratory, Centre of Basic Research II, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece and Laboratory of Biology, University of Athens School of Medicine, Athens, Greece (Greece)

Background / Objectives

Both HPV positive and HPV negative cervical cancers are associated with cell cycle disruption; however, the contribution of the individual oncogenic drivers in cervical cancer is elusive. Therefore, utilization of informative cell lines with or without the HPV genome, can provide insights on these mechanisms. The goal of the current study was to characterize the metabolomic profiles of four distinct cervical cell lines and identify biochemical similarities and differences, reflecting specific pathways of carcinogenesis.

Methods

A normal cervical (HCK1T) and three cervical cancer cell lines, one HPV negative (C33A), and two HPV positive (SiHa HPV16+) and HeLa HPV18+) were used. Metabolites were detected utilizing a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution-accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Welch's two-sample t-test was used to identify biochemicals that differed significantly

Results

The dataset documented a total of 462 biochemicals, 439 known compounds and 23 of unknown structural identity. An altered carbohydrate metabolism was documented in the two HPV-positive cell lines exhibiting features of the Warburg metabolism. This is consistent with the role of HPV E6 in promoting Warburg metabolism, by stabilizing

expression of the glycolytic mediator HIF-1. SiHa and HeLa cells exhibited purine salvage pathway activity, while C33A cells uniquely showed accumulation of cytidine, through a novel mechanism. SiHa and C33A cells exhibited similar profiles in the Cysteine/Glutathione pathway. Analysis of lipid levels revealed distinct metabolic profiles among the four cell lines. To identify other associated changes independent from cervical cancer-derived transformed cells, we sorted the dataset by significance of changes of normal HCK1T cells, relative to other cells. This revealed a large number of changes, with striking increases in dipeptide levels. This may represent a unique pathway to provide alternate sources to increase fuel growth. Finally, several of the 23 identified unnamed biochemicals, exhibited discrete profiles in each of the four cell lines, suggesting cell-line specific utilization.

Conclusion

The metabolomic profiling of normal, HPV positive and HPV negative cervical cancer cells provides novel mechanistic insights into cervical carcinogenesis. Collectively, the data reflect highly dynamic differences among the cellular groups that will help elucidate both unique and common aspects of perturbed growth profiles of cervical cancer.

OC 11-14

BENEFICIAL EFFECTS OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL ON CERVICAL EPITHELIZATION, VAGINAL MICROBIOTA AND VAGINAL HEALTH: A PILOT STUDY IN ASYMPTOMATIC WOMEN

J. Cortés¹, F. Losa², S. Palacios³, D. Dexeus⁴

¹Senior Consultant on Gynecologic Oncology. Former President of the Spanish Association of Colposcopy and Cervical Pathology, Palma de Mallorca. (Spain),

²Gynecology and Obstetrics Department, Clínica Sagrada Familia, Barcelona. (Spain),

³Gynecology and Obstetrics Department, Instituto Palacios de

Medicina y Salud de la Mujer, Madrid. (Spain), ⁴Gynecology and Obstetrics

Department, SOMDEX-Clínica Tres Torres, Barcelona. (Spain)

Background / Objectives

The concept of 'vaginal health' as the vaginal state in which the physiological conditions and the vaginal microenvironment and microbiota are not disrupted, has gained increasing interest in recent years and is also being extended to healthy women. Aim of this study was to assess the effect of a 12-day treatment using a vaginal gel based on niosomes containing hyaluronic acid, β -glucan, alpha-glucan oligosaccharide, *Coriolus versicolor*, *Asian centella*, *Azadirachta indica* and *Aloe vera* on vaginal microbiota, cervical epithelization and vaginal health.

Methods

Open-label, prospective pilot study conducted in asymptomatic women in daily practice. Cervical epithelization was evaluated by colposcopy using an ectopy epithelization score (from 5: no ectopy to 1: severe ectopy and bleeding), vaginal microbiota using the VaginaStatus-Diagnostic test (Instiüt für Mikroökologie, Herborn, Germany) and further rated by the investigator using a 5-point Liker scale (from 5: normal to 1: very severe deterioration in which all evaluated species were altered), and vaginal health using the Vaginal Health Index.

Results

In 21 women, a positive effect to improve epithelization of the cervical mucosa, with a mean score of 4.42 at the final visit as compared to 3.09 at baseline (P < 0.0001) (43% improvement). In 10 women, there was a trend of improving of vaginal microbiota status, with a mean score of 4.0 at the final visit vs. 3.3 at baseline (P = NS) (21.2% improvement). In 11 women, the Vaginal Health Index increased from 19.0 at baseline to 22.3 at the final visit (P = 0.007) (17.3% improvement). The concentration of Lactobacillus spp. increased 54.5% of women and pH decreased from 4.32 to 4.09.

	Baseline	Final	Mean improving	p value
Cervix epithelization score* (n=21)	3.09	4.42	43%	<0.0001
Vaginal Microbiota status* (n=10)	3.3	4.0	21.2%	ns
Vaginal Health Index** (n=11)	19.0	22.3	17.3%	0.007

* see methods to scale

values **Bachmann Vaginal Health Index

Conclusion

In a pilot study conducted among asymptomatic women, the use of a vaginal gel based on *Coriolus versicolor* and other ingredients such as niosomes containing hyaluronic acid, β -glucan, alpha-glucan oligosaccharide, *Asian centella*, *Azadirachta indica* and *Aloe vera* for 12 consecutive days showed positive effects toward improving vaginal microbiota, cervical epithelization and vaginal health. Further studies must be conducted to confirm these positive results, as well as to evaluate the potential use in human papilloma virus infection.

OC 11-15

CERVICAL MICROBIOTA AS A POSSIBLE MODULATOR OF THE CYTOKINE PROFILE AT THE CERVICAL MICROENVIRONMENT IN CERVICAL LESIONS AND CERVICAL CANCER

K.J. Torres-Poveda¹, A. Audirac-Chalifour¹, M. Bahena-Román¹, J. Téllez-Sosa¹, J. Martínez-Barnetche¹, K. Delgado-Romero², A. García-Carrancá³, V. Madrid-Marina³

¹Chronic Infectious Diseases and Cancer Division. Instituto Nacional de Salud Pública (Mexico), ²CAPASAM. Health Services of the State of Morelos (Mexico), ³Division of Basic Research. Instituto Nacional de Cancerología (Mexico)

Background / Objectives

Background. The cervical cancer (CC) is given by HPV persistence due to the immunosuppressive tumor microenvironment generated by immunosuppressive cytokines¹. It is known that the vaginal microbial ecosystem and the cytokine profile play a role in promoting cervical dysplasia². Objectives. We assessed the association between cervical microbiota diversity and composition according to histopathological diagnosis of each stage of the natural history of CC, and evaluated IL-4, IL-6, IL-10, TGF- β 1, TNF- α and IFN- γ mRNA expression levels in cervix across histopathological diagnosis and specific bacterial clusters.

Methods

Methods. We determined cervical microbiota by High-throughput sequencing of 16S rDNA amplicons and classified it in community state types (CST). Mean difference analyses between alpha-diversity and histopathological diagnosis were evaluated. β -diversity analysis within histological diagnosis was carried out. Cytokine mRNA expression at the cervix level was analyzed across CST and histopathological diagnosis.

Results

Results. We found a significant difference of microbiota diversity between non cervical lesions (NCL-HPV) negative vs cervical lesions (CL) and CC ($p=0.006$, $p=0.036$). When β -diversity was evaluated, the CC samples had the highest variation within groups ($p<0.0006$) and largest distance compared to NCL-HPV negative ($p<0.00001$). Predominant bacteria in women with normal cytology are *L. crispatus*

and *L. iners*; *Sneathia* spp. in SIL and *Fusobacterium* spp. in CC. We found higher median levels of IL-4 and TGF- β 1 mRNA at the cervix level, in CST dominated by *Fusobacterium* spp.

Conclusion

Conclusion. These results show that cervical microbiota may play a role in cervical cancer pathology and that HPV infection and the development of CL and CC are associated with changes in microbiota diversity and with cytokine expression patterns at the cervix level.

References

References. (1. Alcocer-González JM, Berumen J, Tamez-Guerra R, Gariglio P, Hernández-Pando R, Bermúdez-Morales VH, et al. In Vivo Expression of Immunosuppressive Cytokines in Human Papillomavirus-Transformed Cancer Cells. *Viral Immunology* 2006;19(3):481-91. 2. Behbakht K, Friedman J, Heimler I, Aroutcheva A, Simoes J and Faro S. Role of the vaginal microbiological ecosystem and cytokine profile in the promotion of cervical dysplasia: A case-control study. *Infect Dis Obstet Gynecol* 2002;10:181-6.)

OC 11-16

Cervical Antimicrobial Peptides Are Decreased Following Excisional Treatment for Cervical Intraepithelial Neoplasia

A. Mitra¹, **M. Paraskevasidi**¹, **I. Kalliala**¹, **J. Lai**¹, **D. Lyons**², **P. Bennett**¹, **S. Stock**³, **K. Maria**¹

¹Institute of Reproductive & Developmental Biology, Imperial College London (United kingdom), ²Department of Obstetrics & Gynaecology, Imperial College Healthcare NHS Trust (United kingdom), ³The Queen's Medical Research Institute, Edinburgh University (United kingdom)

Background / Objectives

Excisional treatment for cervical intraepithelial neoplasia (CIN) increases the risk of preterm birth (PTB) in a subsequent pregnancy. Treatment may disturb the innate immune system, which includes the production of natural antimicrobial peptides by the cervical epithelium. Human Beta Defensin-1 (hBD-1) has potent antiviral, as well as antibacterial activity, and Secretory Leucocyte Protease Inhibitor (SLPI) is found at high levels in the mucus plug of a healthy pregnancy. We examined the effect of antimicrobial peptides in response to excisional treatment.

Methods

Levels of hBD-1 and SLPI were determined in cervicovaginal secretions using enzyme-linked immunosorbent assay (ELISA) and normalized to total protein content.

Results

Two-hundred and seventy nine women with CIN and normal controls were sampled. Mean AMP ratios were lowest in healthy controls, rising with increasing disease severity (hBD-1: normal 4962pg/ml vs high-grade CIN 7173pg/ml ($p=0.038$); SLPI: normal 806422pg/ml vs high-grade CIN 850075pg/ml ($p=0.123$).

Eighty women were sampled 6 months following excision treatment. Levels of hBD-1 were significantly lower compared to pre-treatment (9073pg/ml vs 3838pg/ml, $p<0.0001$) and were also lower than healthy, untreated controls ($p=0.0143$). SLPI levels also decreased in the same manner (137800pg/ml vs 99270pg/ml), but this was not statistically significant.

Conclusion

Excisional treatment leads to a reduction in the levels of antimicrobial peptides, which serve as a first-line defense against pathogens. Treated women may therefore be more susceptible to ascending infections in pregnancy, known to be a significant cause of PTB.

OC 11-17

Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions

L. De Strooper¹, C. Meijer¹, J. Berkhof², A. Hesselink¹, P. Snijders¹, R. Steenbergen¹, D. Heideman¹

¹Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands (Netherlands), ²Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands (Netherlands)

Background / Objectives

Primary testing for human papillomavirus (HPV) in cervical screening requires triage to differentiate women with transient infection from those with persistent infection who require more intensive management given their risk for cervical (pre)cancer. In this study, the clinical performance of a novel methylation marker *FAM19A4* for the triage of high-risk (hr)HPV-positive women was evaluated.

Methods

Using a training-validation set approach, we analyzed a *FAM19A4* quantitative methylation-specific PCR (qMSP). The validation set comprised hrHPV-positive cervical scrapes of 43 women with cervical intraepithelial neoplasia grade 3 or worse (CIN3+) and 135 women with \leq CIN1. The validation set comprised hrHPV-positive cervical scrapes of 52 women with CIN2+, including 33 CIN3+, 19 CIN2, and 166 women with \leq CIN1.

Results

The methylation threshold of *FAM19A4* qMSP that gave rise to CIN3+ specificity of 70% in the validation set was applied in the validation set. This resulted in CIN3+ sensitivity of 75.8% [95% confidence interval (CI): 61.1-90.4] at 67.0% (95% CI: 60.3-73.8) specificity. Next, the validated qMSP was applied to an independent series of hrHPV-positive cervical scrapes of 22 women with cervical cancer, 29 with advanced CIN2/3 [i.e., women with a known preceding hrHPV infection (PHI) lasting \geq 5 years as proxy of longer duration of lesion existence], and 19 with early CIN2/3 (i.e., PHI $<$ 5 years). All carcinomas (22/22) and advanced CIN2/3 lesions (29/29) were *FAM19A4* methylation-positive, compared with 42.1% (8/19; 95% CI: 19.9-64.3) of early CIN2/3 lesions.

Conclusion

FAM19A4 is an attractive triage marker for hrHPV-positive women, with a high reassurance for the detection of cervical carcinoma and advanced CIN2/3 lesions.

OC 11-18

Tumor specific imaging in HPV16 positive cervical cancer using HPV16-E7 binding affibody molecules

L. Zhang, X. Xue, W. Du, L. Wang, Y. Xiong, C. Zhang, Y. Song, Y. Cai, D. Cen, P. Jiang, K. Zhao

Department of Microbiology and Immunology, Institute of molecular virology and immunology, Wenzhou Medical University, Wenzhou, China (China)

Background / Objectives

Cervical cancer caused by infection with high-risk human papillomavirus remains to be the most deadly gynecologic malignancy worldwide. It is well documented that persistent expression of two oncogenes (E6/E7) plays the key roles in viral-induced cancers. Thus, in vivo detection of the oncoproteins is very important for early diagnosis and prevention of the cancer, especially, the invasive cancer. Recently, affibody molecules have been demonstrated to be a powerful targeting probe for tumor-targeted imaging and diagnosis.

Methods

In this study, four HPV16 E7-binding affibody molecules (ZHPV16E7:127, ZHPV16E7:301, ZHPV16E7:384 and ZHPV16E7:745) were screened from a phage-displayed peptide library and used for molecular imaging in tumor-bearing mice.

Results

Biosensor binding analyses showed first that the four affibody molecules bound to HPV16E7 with very high affinity and specificity. They co-localized with E7 protein only in two HPV16-positive cancer cells (SiHa and CaSki). Furthermore, affibody ZHPV16E7:384 was conjugated with Dylight755 and used for in vivo tumour-imaging. Strongly high-contrast tumor retention of this affibody only occurred in HPV16-derived tumors of mice as early as 30 min post-injection, not in HPV-negative and HPV18-derived tumors. The accumulation of Dylight755-conjugated ZHPV16E7:384 in tumor was achieved over a longer time period (24 h).

Conclusion

The data provide strong evidence that E7-specific affibody molecules have great potential used for molecular imaging and diagnosis of HPV-induced cancers.

References

This work was supported by the National Nature Science Foundation of China (81172463), the Zhejiang Provincial Natural Science Foundation of China (LY15H190009) and Wenzhou Science and Technology Bureau of China (Y20140659).

OC 12-01

HPV PREVALENCE AMONGST DANISH HPV SELF-SAMPLING WOMEN STRATIFIED BY SCREENING HISTORY AND COMPARED TO WOMEN UNDERGOING ROUTINE SCREENING.

H. Pedersen¹, J.U.H. Lam¹, M. Rebolj², D.M. Ejegod¹, L. Thomsen³, S.K. Kjaer⁴, S. Preisler¹, C. Rygaard¹, E. Lynge⁵, J. Bonde⁶

¹Department of Pathology, Copenhagen University Hospital Hvidovre, Hvidovre (Denmark), ²Clinical Research Centre, Copenhagen University Hospital Hvidovre, Hvidovre (Denmark), ³Danish Cancer Society, Unit of Virus, Lifestyle and Genes, Copenhagen (Denmark), ⁴Danish Cancer Society, Unit of Virus, Lifestyle and Genes, Copenhagen; Department of Obstetrics and Gynecology, Copenhagen University Hospital Rigshospitalet, Copenhagen (Denmark), ⁵Department of Public Health, University of Copenhagen, Copenhagen (Denmark), ⁶Department of Pathology, Copenhagen University Hospital Hvidovre, Hvidovre; Clinical Research Centre, Copenhagen University Hospital Hvidovre, Hvidovre (Denmark)

Background / Objectives

To increase the participation in the cervical screening program in the Capital Region of Denmark, HPV-based self-sampling was offered free of charge as an opt-in strategy to screening non-attenders. Here, we compared the HPV prevalence and genotype distribution between self-sampling participants and the women who regularly participate in screening. To increase the participation in the cervical screening program in the Capital Region of Denmark, HPV-based self-sampling was offered free of charge as an opt-in strategy to screening non-attenders. Here, we compared the HPV prevalence and genotype distribution between self-sampling participants and the women who regularly participate in screening.

Methods

Women from the Capital Region who were not screened in the last screening round or longer (N= 54,585) and their screening history were identified from the invitational module of the nationwide Pathology Data Bank. HPV prevalence was determined by using the CLART HPV2 assay (Genomica, Madrid, Spain). Screening history was categorized as intermittently screened (screened within the last 10 years but not within the last screening round) or long-term unscreened (not screened in the last 10 years or more). The results reported herein are based on the 4823 self-sampling

brushes from 23,632 invited women. Data for regularly screened women are from the Danish Horizon study.

Results

Twenty-three samples (0.5%) had an invalid result on the CLART assay. The overall HR-HPV prevalence in the study was 11.3%, with 11.2% in intermittently screened women, and 11.4% in long-term unscreened women. In comparison, routinely screened women had an HPV prevalence of 15.8%. In intermittently screened women, the most prevalent high-risk HPV genotypes were 16, 51, 31, and 52 (8.1%, 6.2%, 4.9%, and 4.7%, respectively), whereas the most prevalent genotypes in long-term unscreened women were 16, 51, 52, and 31 (9.1%, 6.3%, 4.6%, and 4.3%, respectively). Among women undergoing routine screening, the most prevalent HPV types were 16, 52, 31 and 58 (8.8%, 6.5%, 5.8% and 5.1% respectively). HPV61 was the most prevalent low-risk HPV genotype in both the intermittently screened and long-term unscreened women, with 10.5% and 11.4%, respectively, whereas among regularly screened women this was HPV53 with 7.3%.

Conclusion

Differences in HPV prevalence and genotype frequency between under-screened women participating in self-sampling and regularly screened women were observed, though the differences were small.

OC 12-02

HPV GENOTYPE DISTRIBUTION AMONG WOMEN WITH \geq CIN2: COMPARISON OF PRIMARY SCREENED WOMEN AND UNDER-SCREENED WOMEN OFFERED SELF-SAMPLING.

L. De Thurah¹, J.U.H. Lam¹, M. Rebojl², S. Preisler³, E. Lyngø⁴, C. Rygaard¹, D. Ejegod¹, J. Bonde³

¹Department of Pathology, Copenhagen University Hospital Hvidovre (Denmark), ²Clinical Research Centre, Copenhagen University Hospital Hvidovre (Denmark), ³Department of Pathology/ Clinical Research Centre, Copenhagen University Hospital Hvidovre (Denmark), ⁴Department of Public Health, Center for Epidemiology and Screening, University of Copenhagen (Denmark)

Background / Objectives

In primary cervical cancer screening, the follow up after a positive test for high-risk Human Papilloma Virus (hrHPV) is fairly well established, whereas this is not the case for women who do not attend primary screening and alternatively are offered screening by self-sampling. Looking at \geq CIN2 cases, we here compare the distribution of hrHPV genotypes in under-screened women who were screened through HPV self-sampling (Copenhagen Self Sampling Initiative, CSi, preliminary data) to that of women attending primary screening (The Danish Horizon study). Based on genotype, could some of these women benefit from direct referrals to colposcopy rather than referral to follow up cytology?

Methods

From both studies, we included women aged \geq 30 years (Horizon: N=2974, CSi: N=4440). Women with a positive hrHPV test at baseline (Horizon N=461, CSi N=462) and a subsequent histological diagnosis of \geq CIN2 were compared by retrieving genotype result from the baseline sample. The follow up periods for Horizon is 3 years at present, while follow up currently is up to 12 months for CSi. All HPV DNA testing was undertaken using the CLART HPV2 assay (Genomica, Spain). \geq CIN2 cases were identified through passive follow-up in the Danish Pathology Data Bank.

Results

Fifty \geq CIN2 cases were identified among primary screened women, corresponding to 10.8% of hrHPV positive women in the group. Among those offered HPV self-sampling 58 \geq CIN2 cases were detected, equal to 12.6% of hrHPV positive cases. The mean ages of the women included in the analysis were 39 (\pm 10.5) and 44 (\pm 10.6) years, for Horizon and CSi respectively. One-third of the under-screened CSi women were above the age of 50, with only 10% being above the age of 50 among the Horizon women. HPV16 was the most frequent genotype observed in the baseline sample with 23% (Horizon) and 34% (CSi). HPV52 and HPV31 were the 2nd and 3rd most prevalent genotypes. For under-screened women offered self-sampling, HPV16, 52 and 31 accounted for 56% of all observed \geq CIN2. The frequency of the remaining 10 hrHPV genotypes differed considerably between the two groups.

Conclusion

Although based on small numbers, HPV16, HPV52 and HPV31 were the most prevalent genotypes in both groups. Primary screened women with \geq CIN2 were younger compared to under-screened women offered self-sampling, however this is an effect of women not participating in primary screening on average being older. The high frequency of HPV16, HPV52 and HPV31 leading to \geq CIN2, and the women's status as under-screened lead us to propose that under-screened women accepting self-sampling and found positive for any of these genotypes should be directly referred for gynaecology examination and colposcopy.

OC 12-03

ACCESSING*: SELF-SAMPLING AND HPV ONCOPROTEIN TESTING COMBINED WITH GENO-TYPING FOR HPV EPIDEMIOLOGY IN RURAL SETTINGS.

A. Krings¹, S. Tetteh², R. Adaletey², P. Dunyo², B. Hansen², C. Wormenor², I. Gedzah², J. Amuah³, A. Pesic¹, G. Akwada², A.M. Kaufmann¹

¹Clinic for Gynecology, Charité Universitätsmedizin Berlin, Germany (Germany), ²Catholic Hospital Battor, Volta Region, Ghana (Ghana), ³School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa, Canada (Canada)

Background / Objectives

Epidemiology of HPV infection in rural settings is more difficult to investigate than in more accessible urban areas. A comprehensive impression and information on HPV prevalence also in such locations is important. Self-sampling may be a more acceptable method for material collection and less trained personnel could support it. HPV testing on cervicovaginal brush samples should result in data on circulating HPV genotypes, age of highest prevalence, mean prevalence and should be correlated to risk for disease.

Methods

2000 women between the age of 18 and 65 years from rural communities in the North Tongu District in Ghana, representatively selected from the geographical area and socioeconomical setting, self-collected cervico-vaginal samples using the Evalyn brush device. Samples were transported in the absence of collection media to the lab within 7 days, transferred into methanol-based cell preservation medium and kept at 4°C. From the cell suspension Arbor Vita OncoE6™ Cervical Test, a molecular lateral flow assay for detection of HPV16 and 18 E6 protein, was performed followed by full genotyping using GP5+/6+ PCR with Luminex read-out.

Results

Sample collection in rural communities was highly successful. 2000 samples were collected by Evalyn brush and acceptability was very high. The median age of women screened was 30 years. The OncoE6 Cervical test results revealed a

positivity rate of 2% (41/1988) with 5 HPV16+, 11 HPV18+ and 25 HPV16/18+ samples. Initial results present much higher positivity by HPV genotype testing. Concordance with HPV genotyping results and clinical confirmation will be evaluated once all results are available. Follow-up of the patients will be done by PAP smear and colposcopy where indicated due to HPV positivity.

Conclusion

The initial results imply that a molecular triage test may be important to reduce referrals after HPV testing especially in settings with limited resources.

OC 12-04

Age-specific hrHPV detection using cervical, vaginal and urine samples of women attending routine cervical screening. One sample doesn't fit all?

G.A. Stanczuk¹, **G. Baxter**², **C. Heather**³, **F. William**³, **C. Kate**⁴, **W. Allan**⁵, **A. Marc**⁶

¹Dept. of Obstetrics and Gynaecology, Western Isles Hospital, Stornoway, SH1 4AF (United kingdom), ²Dept. of Research and Development, Dumfries and Galloway Royal Infirmary, Bankend Rd, Dumfries, DG1 4 AP (United kingdom), ³Dept. of Obstetrics and Gynaecology, Dumfries and Galloway Royal Infirmary, Bankend Rd, Dumfries, DG1 4 AP (United kingdom), ⁴Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh, Edinburgh, (United kingdom), ⁵Dept. of Pathology, Monklands Hospital, Airdrie. (United kingdom), ⁶Unit Cancer Epidemiology, Scientific Institute of Public Health, Brussels. (Belgium)

Background / Objectives

We previously reported PaVDAg study, which demonstrated that cobas 4800 hrHPV testing on self-collected vaginal samples have similar sensitivity and specificity for detection of CIN2+ as on clinician-collected cervical samples. The relative sensitivity of cobas testing on random urine versus clinician samples was 0.67 hence could not be considered for primary cervical screening before further analytical optimisation. Here we are presenting the age-specific hrHPV detection in clinician-collected cervical and two self-collected samples.

Methods

A total of 5318 women aged 20-60 attending for routine cervical screening provided clinician-collected cervical, self-collected urine and vaginal samples for hrHPV testing. HrHPV testing of all samples was performed using the cobas 4800. We analysed hrHPV detection in urine and vaginal samples vs. cervical samples in increasing age groups of participants.

Results

The proportion of hrHPV positive results was similar in cervical and vaginal samples in women ≤ 29 years old. Comparatively, hrHPV positivity was 38% higher in vaginal compared to cervical samples in women ≥ 50 years. This was also reflected in HPV16/18 positivity, which was 26% higher in vaginal samples compared with cervical samples in women ≥ 50 years old. The increase of HPV positivity ratio from

1.01 to 1.38 with increasing age was significant ($p=0.03$). Similarly the ratios of hrHPV positivity in urine vs. cervical samples increased from 0.66 to 0.95, with significant linear trend $p=0.02$. The significant differences in hrHPV detection between vaginal vs. cervical samples was already observed in women 45 years and older.

Conclusion

We hypothesise that the quality of cervical sampling deteriorates around the menopause and that menopausal atrophic changes in urogenital mucosa contribute to an increase of unsatisfactory cytology and a lower content of HPV DNA in cervical samples. These changes appear to be less influential of self-collected samples. Given clinical performance of vaginal samples we cannot rule out the possibility that, in follow-up, relatively more CIN2+ may be detected in older women who were hrHPV+ on the self-sample alone. Another hypothesis is that specificity of HPV testing on self-samples deteriorates with increasing age. Future longitudinal analysis of the PaVDaG cohort will provide some answers.

OC 12-05

CERVICAL SCREENING IN RURAL MALAWI USING XPERT® HPV AND SELF-TAKEN VAGINAL SAMPLES

H. Cubie¹, E. Kawonga², M. Mautanga², I. Mwenitete², B. Kabota³, D. Morton³, R. Ter Haar³, C. Campbell⁴, G. Stanczuk⁵

¹Global Health Academy, University of Edinburgh (United kingdom), ²Nkhoma Hospital Laboratory, Nkhoma, Malawi (Malawi), ³Nkhoma CCAP Hospital, Nkhoma, Malawi (Malawi), ⁴Usher Institute for Populations Health Sciences and Informatics, University of Edinburgh (United kingdom), ⁵NHS Highland and GHA, University of Edinburgh (United kingdom)

Background / Objectives

To test whether self-taken vaginal specimens could be used with Xpert® HPV for primary screening prior to visual inspection with acetic acid (VIA) in Malawi

To compare HPV prevalence in self-taken versus clinician-collected cervical samples using Xpert® HPV.

Methods

Self-taken samples were obtained from women attending routine VIA clinics in Nkhoma Hospital, using cotton swabs suspended in 5ml of PreservCyt® solution. All samples were tested in the local laboratory using the Xpert HPV assay. Results were compared with Xpert® HPV results from 750 clinician-collected LBC samples in PreservCyt®, obtained from women attending the same clinics in the preceding year.

Results

Overall HPV positivity in the LBC samples was 19.9%, compared with 24.5% in the self-taken samples. Of positive detections, HPV 16 and HPV 18/45 accounted for 24.2% each and HR-HPV 'others' for 64.4% in LBC, compared with 17.7%, 26.5% and 76.1% respectively in the self-taken samples. Multiple infections were also more frequently detected in self-taken samples. HPV prevalence in known HIV positive women was comparable (43.4% in LBC ; 45% in vaginal samples).

Conclusion

Self-taken vaginal samples using cotton swabs gave valid results with Xpert HPV and showed higher HPV positivity than clinician-collected cervical samples. HPV 16 was less frequently detected in vaginal samples, while HPV 18/45, 'others' and multiple infections were more common. Self-sampling was well accepted and satisfactorily collected by women. Sending only HR-HPV positive women to VIA would significantly reduce the burden on clinical staffing resources and skills.

OC 12-06

VALIDATION OF A NEW HPV SELF-SAMPLING DEVICE: THE CERVICAL AND SELF-SAMPLE IN SCREENING (CASSIS) STUDY

M. El-Zein¹, **S. Bouten**¹, **K. Louvanto**¹, **L. Gilbert**², **W. Gotlieb**³, **R. Hemmings**⁴, **M. Behr**⁵, **E. Franco**¹

¹Division of Cancer Epidemiology, McGill University (Canada), ²Gynecologic Cancer Service, McGill University Health Centre - Glen Site Cedars Cancer Centre (Canada), ³Division of Gynecologic Oncology and Colposcopy, McGill University - Jewish General Hospital (Canada), ⁴Department of Obstetrics and Gynecology, McGill University Health Centre - St Mary's Hospital Centre (Canada), ⁵Department of Microbiology, McGill University Health Centre (Canada)

Background / Objectives

Vaginal self-sampling for human papillomavirus (HPV) testing can increase the coverage of cervical cancer screening programs. Our primary objective was to compare the diagnostic self-sampling performance of the newly designed HerSwab™ device (Eve Medical Inc.) with a physician-collected cervical sample for the detection of cervical intraepithelial neoplasia (CIN) and cancer. We also compared the performance of HerSwab™ with the cobas® PCR Female swab.

Methods

Women (n=463) referred for colposcopy at McGill University affiliated hospitals collected two consecutive self-samples with the HerSwab™ and cobas® swabs, after receiving written and oral instructions. Randomization determined which swab was first. The colposcopist then collected a cervical sample and conducted a standard colposcopic examination with biopsies as appropriate. The HerSwab™ and physician-collected specimens were transferred to individual ThinPrep-containing vials. The cobas® swabs were suspended in cobas®PCR media. All samples were genotyped using the Roche's cobas® 4800 HPV system. Agreement between collection methods was measured using the Kappa (k) statistic. Sensitivity and specificity and their respective 95% confidence intervals (CI) were calculated for the three HPV test results based on histological grades.

Results

Of the 463 women, 319 (68.9%) were classified as normal, 65 (14.0%) had CIN1, 31 (6.7%) had CIN2, 46 (9.9%) had CIN3 and 2 (0.4%) women had adenocarcinoma. We found very good agreement of HPV detection between the screening methods (HerSwab™ and physician: $k=0.89$ for HPV16, $k=0.88$ for HPV16/18, $k=0.81$ for any high-risk HPV; HerSwab™ and cobas® swabs: $k=0.92$ for HPV16, $k=0.89$ for HPV16/18, $k=0.85$ for any high-risk HPV). Sensitivity and specificity of any high-risk HPV for CIN2+ by HerSwab were 83.5% (95% CI: 75.4-91.7) and 52.9% (95% CI: 47.9-57.9), respectively. Corresponding values were 83.5% (95% CI: 75.4-91.7) and 48.4% (95% CI: 43.4-53.4) for the cobas® swab, and 91.1% (95% CI: 84.9-97.4) and 52.9% (95% CI: 47.9-57.9) for physician-collected samples.

Conclusion

Results from CASSIS demonstrate that HerSwab™ has a good agreement with physician sampling in detecting HPV genotypes, and adequate performance in detecting high-grade lesions. Its use could overcome some of the barriers to cytology, promoting cervical cancer prevention among unscreened and under-screened women.

OC 12-07

EVALUATION OF HIGH RISK HPV DNA DETECTION IN SELF-COLLECTED VAGINAL SAMPLES AND URINE FROM A TEST-OF-CURE SETTING

S. Andersson, M. Mints, E. Östensson

Karolinska Institutet, Department of Women's and Children's Health (Sweden)

Background / Objectives

To compare the high-risk (hr) HPV status of self-collected vaginal fluid and first void urine with that of physician-sampled cervical scrapes (CS) collected at the same visit 6 months post first life-time treatment of high grade CIN from 500 women using a clinically validated PCR-based test for cervical cancer screening and assesses correlation of results to post-treatment cytology.

Methods

Triplets of pre-cytology physician-sampled CS (PreservCyt LBC, Hologic), vaginal fluid (Qvintip, Aprovix) and urine were collected from 200 women enrolled at date (pre-treatment histology: 45 CIN2, 145 CIN3, 6 CIN3/AIS, 4 AIS; 30% with unclear cone margins). Qvintip brush heads (air dried; stored at room temperature) were transferred into Cervi-Collect Tubes (Abbott) prior to testing. Urine samples were transferred into Cervi-Collect Tubes within 30 minutes from collection and stored frozen until testing. HrHPV DNA testing was performed with the clinically validated RealTime High Risk HPV assay (Abbott) using the manufacturer's standard protocol on the m2000 System. RealTime High Risk HPV is a multiplex real-time PCR detecting 14 hrHPV types and simultaneously differentiating HPV16 and HPV18 from 12 non-HPV 16/18 types; its internal control identifies a human β -globin sequence to ensure sample cellularity.

Results

Valid test results were obtained from all physician-sampled CS samples and the vast majority of self-collected specimens (Qvintip: 199; urine: 192). Evaluation of results from 191 matched triplets available for evaluation revealed high overall-concordance of hrHPV DNA test results between self-and physician-sampled specimens (Qvintip 88.0%, $k=0.7$ urine 90.1%, $k=0.7$ and across ascending cytological categories (N=155 WNL: Qvintip 87.1%, urine 91.6%; N=27 LSIL: Qvintip 92.6%, urine 81.5%; N=9 HSIL: Qvintip and urine 88.9%). Partial typing pattern in women with HSIL were almost identical among all three sample types, while a trend towards higher

discrepancy of partial typing pattern was observed in women with LSIL and WNL, the latter mainly driven by the presence of non-HPV16/18 in self-collected materials.

Conclusion

Preliminary results from this study show good correlation of hrHPV DNA results from self-collected vaginal fluid and urine with those from physician-sampled CS collected in a test-of-cure setting. Self-collection may have potential for post-surgical follow up of high-grade cervical lesions and support reducing unnecessary procedures on healthy women by physicians or midwives. However, larger studies are required to confirm these findings based on post-treatment outcome and evaluate potential limitations of the approach.

OC 12-08

FIRST-VOID URINE AND PHYSICIAN-TAKEN SMEAR SHOW SIMILAR SENSITIVITY FOR THE DETECTION OF CIN2+ LESIONS

A. Leeman¹, **M. Del Pino**², **A. Rodriguez**², **V. Vankerckhoven**³, **K. Beyers**³, **M. De Koeijer**³, **M. De Koning**¹, **A. Torne**², **J. Ordi**⁴, **W. Quint**¹

¹DDL Diagnostic Laboratory, Visseringlaan 25, 2288 ER Rijswijk (Netherlands), ²Institute of Gynecology, Obstetrics and Neonatology, Hospital Clínic Clínic— Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona (Spain), ³Novosanis, Bijkhoevelaan 32c, BE-21220 Wijnegem (Belgium), ⁴Department of Pathology, Hospital Clínic, Barcelona (Spain)

Background / Objectives

To study the correlation between HPV detection using self-collected urine samples, brush-based self-samplers and physician-taken smears and to compare HPV detection in morning first-void urine to HPV detection in first-void urine from any hour during the day.

Methods

Women referred to a colposcopy clinic after an abnormal PAP-smear result were sent a device (Colli-Pee™, Novosanis, Wijnegem, Belgium) to collect first void-urine on the first urine of the morning of their colposcopy. A second first-void urine sample was collected during the visit, together with a physician-taken cervix smear, a brush-based self-sample (Evalyn brush™, Rovers Medical Devices B.V., Oss, The Netherlands) and a colposcopy directed biopsy. Urine samples, smear and brushes were tested with the analytically sensitive SPF10-DEIA-LiPA25version1 assay and the clinically validated GP5+/6+-EIA, with LMNX genotyping. Histological material was assessed by a local pathologist.

Results

Morning (U1) and afternoon (U2) urine samples, physician-taken smear (PTS) and brush based self-samples (SS) from 91 patients were analysed. All CIN3 lesions (N=6) were hrHPV positive in PTS, SS, U1 and U2 with both the SPF10-assay and the GP5+/6+-assay (Table 1).

The sensitivity for CIN2+ detection in PTS, SS, U1 and U2 with the SPF10 was 96.4%, 92.9%, 92.9% and 96.4% respectively ($p>0.05$). With the GP5+/6+ assay, the sensitivity was 78.6% in PTS, 82.1% in SS, 92.9% in U1 and 85.7% in U2 ($p>0.05$).

On genotype level, a substantial to almost excellent agreement was found between all samples (kappa 0.663-0.912), for both SPF10 and GP5+6+. When comparing the genotypes found in PTS to those found in urine samples, 12.6% of the samples were discordant when tested with SPF10 and 19.8% of the samples when tested with GP5+/6+. For the comparison between SS and urine, discordant results were found in 10.0% and 14.3% of the samples, respectively. When comparing PTS to SS, 11.0% of the samples were discordant with SPF10 and 16.5% with GP5+/6+. Between U1 and U2, 3.2% were discordant with SPF10 and 6.6% with GP5+/6+.

Table 1: Positivity for SPF10 and GP5+/6+ assay in physician taken smear(PTS), brush-based self-sample (SS), morning first-void urine (U1) and first-void urine from any hour of the day (U2)

Histological diagnosis	PTS	PTS	SS	SS	U1	U1	U2	U2
	SPF10	GP5+/6+	SPF10	GP5+/6+	SPF10	GP5+/6+	SPF10	GP5+/6+
Negative (46)	56.6%	47.8%	56.5%	50.0%	58.7%	45.7%	63.0%	52.2%
CIN1 (17)	82.4%	82.4%	82.4%	76.5%	82.4%	76.5%	82.4%	70.6%
CIN2 (22)	95.5%	72.7%	90.9%	77.3%	90.9%	90.9%	95.5%	81.8%
CIN3 (6)	100%	100%	100%	100%	100%	100%	100%	100%
<i>Total</i>	<i>67</i>	<i>58</i>	<i>66</i>	<i>59</i>	<i>60</i>	<i>60</i>	<i>70</i>	<i>60</i>

Conclusion

CIN2+ detection using HPV testing in first-void urine seems feasible, with sensitivity similar to the sensitivity of the physician-taken smears and brush-based self-samples. No advantage in testing morning first-void urine over first-void urine from later during the day was found.

OC 12-09

THE CLINICAL VALUE OF HPV GENOTYPING IN TRIAGE OF WOMEN WITH HIGH-RISK-HPV-POSITIVE SELF-SAMPLES

R. Ebisch¹, G. De Kuyper-De Ridder¹, R. Bosgraaf¹, L. Massuger¹, J. Inthout², V. Verhoef³, D. Heideman³, P. Snijders³, C. Meijer³, F. Van Kemenade⁴, J. Bulten⁵, A. Siebers⁵, R. Bekkers⁶, W. Melchers⁷

¹Department of Obstetrics and Gynecology, Radboud university medical center Nijmegen (Netherlands), ²Institute for Health Sciences, Radboud university medical center Nijmegen (Netherlands), ³Department of Pathology, VU University Medical Center (Netherlands), ⁴Department of Pathology, Erasmus MC, University Medical Center (Netherlands), ⁵Department of Pathology, Radboud university medical center (Netherlands), ⁶Department of Obstetrics and Gynecology, Radboud university medical center (Netherlands), ⁷Department of Medical Microbiology, Radboud university medical center (Netherlands)

Background / Objectives

Cytology alone, or combined with HPV16/18 genotyping, might be an acceptable method for triage in hrHPV-cervical cancer screening. Previously studied HPV-genotype based triage algorithms are based on cytology performed without knowledge of hrHPV status. The aim of this study was to explore the value of hrHPV genotyping combined with cytology as triage tool for hrHPV-positive women.

Methods

520 hrHPV-positive women were included from a randomized controlled self-sampling trial on screening non-attendees (PROTECT-3B). Eighteen baseline triage strategies were evaluated for cytology and hrHPV genotyping (Roche Cobas 4800) on physician-sampled triage material. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), referral rate, and number of referrals needed to diagnose (NRND) were calculated for CIN2+ and CIN3+. A triage strategy was considered acceptable if the NPV for CIN3+ was $\geq 98\%$, combined with maintenance or improvement of sensitivity and an increase in specificity in reference to the comparator, being cytology with a threshold of atypical cells of undetermined significance (ASC-US).

Results

Three triage strategies met the criteria: HPV16+ and/or \geq LSIL; HPV16+ and/or \geq HSIL; (HPV16+ and/or HPV18+) and/or \geq HSIL. Combining HPV16+ and/or \geq HSIL yielded the highest specificity (74.9%, 95%CI 70.5–78.9), with a sensitivity (94.4%, 95%CI 89.0–97.7) similar to the comparator (93.5%, 95%CI 87.7–97.1), and a decrease in referral rate from 52.2% to 39.5%.

Conclusion

In case of prior knowledge of hrHPV presence, triage by cytology testing can be improved by adjusting its threshold, and combining it with HPV16 /18 genotyping. These strategies improve the referral rate and specificity for detecting CIN3+ lesions, while maintaining adequate sensitivity.

OC 12-10

VALIDATION OF THE FAM19A4/MIR124-2 DNA METHYLATION TEST FOR BOTH LAVAGE- AND BRUSH-BASED SELF-SAMPLES TO DETECT CERVICAL (PRE)CANCER IN HPV-POSITIVE WOMEN

L. De Strooper¹, V. Verhoef¹, J. Berkhof², A. Hesselink³, H. De Bruin¹, F. Van Kemenade⁴, R. Bosgraaf⁵, R. Bekkers⁵, L. Massuger⁵, W. Melchers⁶, R. Steenbergen¹, P. Snijders¹, C. Meijer¹, D. Heideman¹

¹Department of Pathology, VU University Medical Center, Amsterdam (Netherlands), ²Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam (Netherlands), ³Self-Screen B.V., Amsterdam, (Netherlands), ⁴Department of Pathology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (Netherlands), ⁵Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, The Netherlands (Netherlands), ⁶Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands (Netherlands)

Background / Objectives

DNA methylation analysis of cancer-related genes is a promising tool for HPV-positive women to identify those with cervical (pre)cancer (CIN3+) in need of treatment. However, clinical performance of methylation markers can be influenced by the sample type utilized. We describe a multiplex quantitative methylation-specific PCR that targets *FAM19A4* and *mir124-2* loci, to detect CIN3+ using both HPV-positive lavage- and brush self-samples.

Methods

We determined methylation thresholds for clinical classification using HPV-positive training sets comprising lavage self-samples of 182 women (including 40 with CIN3+) and brush self-samples of 224 women (including 61 with CIN3+). Subsequently, independent HPV-positive validation sets of 389 lavage self-samples (including 78 with CIN3+), and 254 brush self-samples (including 72 with CIN3+) were tested using the preset thresholds. Furthermore, the clinical performance of combined methylation analysis and HPV16/18 genotyping was determined.

Results

Training set analysis revealed similar *FAM19A4* and *mir124-2* thresholds for both self-sample types to yield highest CIN3+ sensitivity at 70% specificity. Validation set analysis resulted in a CIN3+ sensitivity of 70.5% (95%CI:60.4-80.6) at a specificity of 67.8% (95%CI:62.7-73.0) for lavage self-samples, and a CIN3+ sensitivity of 69.4% (95%CI:58.8-80.1) at a 76.4% (95%CI:70.2-82.6) specificity for brush self-samples. In combination with HPV16/18 genotyping, CIN3+ sensitivity and specificity were 88.5% (95%CI:81.4-95.6) and 46.0% (95%CI:40.4-51.5) for lavage self-samples, and 84.7% (95%CI:76.4-93.0) and 54.9% (95%CI:47.7-62.2) for brush self-samples.

Conclusion

FAM19A4/mir124-2 methylation analysis performs equally well in HPV-positive lavage- and brush self-samples to identify women with CIN3+. In combination with HPV16/18 genotyping, significantly higher CIN3+ sensitivities are obtained, at decreased specificity.

OC 12-11

VALIDATION STUDY OF SELF-COLLECTED VAGINAL DRY SWABS USING THE XPERT HPV ASSAY FOR HUMAN PAPILLOMAVIRUS DETECTION

R. Catarino¹, **P. Vassilakos**², **A. Bilancioni**¹, **S. Bougel**³, **U. Meyer-Hamme**¹, **P. Petignat**¹

¹Geneva University Hospitals (Switzerland), ²Geneva Foundation for Medical Education and Research (Switzerland), ³Biopath Lab SA (Switzerland)

Background / Objectives

The Xpert® HPV assay (Xpert) offers the opportunity of a point-of-care test to detect high-risk human papillomavirus (HPV) infection. This test is performed with a cervical specimen collected into a vial of PreservCyt transport medium. Our aim was to evaluate whether a vaginal self-sampling with a dry cotton swab for hrHPV detection with the Xpert is a valid alternative to the PreservCyt-collected sample.

Methods

A total of 150 women aged at least 18 years old attending the colposcopy clinic in the University Hospital of Geneva were recruited. Two vaginal specimens were collected for HPV testing and stored in different mediums for each woman. Women firstly self-collected a vaginal sample using a dry cotton swab (s-DRY) and then the physician collected a cervical specimen in PreservCyt (dr-WET). HPV analysis was performed by the Xpert. The remaining sample immersed in PreservCyt was tested for HPV DNA using the cobas test.

Results

HPV positivity was 49.1% for s-DRY, 41.8% for dr-WET and 46.2% for cobas. A good agreement was found between s-DRY and dr-WET samples ($\kappa=0.64$), especially for LSIL+ lesion ($\kappa=0.80$). An excellent agreement was found between the two samples for HPV16 detection in general ($\kappa=0.91$) and among LSIL+ lesion ($\kappa=1.00$). The mean Ct-values were 30.5 ± 5.0 for dr-WET samples and 25.1 ± 14.3 for s-DRY samples ($p<0.001$). S-DRY and dr-WET showed similar sensitivity (79.2%, 78.8%), using cobas results as gold standard. Sensitivities were 84.2% (s-DRY), 73.1% (dr-WET) and 77.8% (cobas) for CIN2+ detection, respectively.

Conclusion

Self-collected vaginal samples with dry cotton swabs are a valid alternative to wet-collected cervical samples for testing with the Xpert HPV assay.

OC 12-12

AGREEMENT OF VAGINAL SELF-SAMPLING AND PHYSICIAN-COLLECTED HPV TEST IN WOMEN ATTENDING A COLPOSCOPY CLINIC IN THAILAND.

N. Phoolcharoen¹, N. Kantathavorn², C. Taepisitpong², G. Sornsamdang², W. Krongthong²

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn university (Thailand), ²Chulabhorn hospital (Thailand)

Background / Objectives

Despite the incidence and mortality worldwide has declined, cervical cancer remains a leading public health problem in Thailand. One of the main reasons not to go to participate in screening program in Thai women is embarrassment. This study evaluated the agreement of vaginal self sampling and physician-collected HPV test and Thai women's acceptability.

Methods

Participants were 250 women age 30-70 years who attended a colposcopy clinic in Chulabhorn hospital, Bangkok, Thailand. Vaginal self specimens were obtained by brush and the women were examined by physician to collect the specimens. All specimens were tested for High risk HPV with the Cobas 4800 HPV test. The acceptability of self-sampling HPV was assessed.

Results

HR HPV was detected in 41.6% of self- and 35.6% of physician-collected samples. Overall, there was 87.6% agreement between self- and physician-collected specimens. The complete concordance was detected in 87.6%, partial concordance in 1.2% and discordance in 11.2%. Over 90% of women satisfied and reported comfortable feeling with self-sampling method.

Conclusion

Self-sampling showed favorable agreement with physician-collected and also high acceptability in Thai women. This method can be used to increase the coverage of cervical screening in Thailand.

OC 12-13

CONFIDENCE™ HPV TEST VALIDATION FOR QVINTIP SELF-COLLECTED VAGINAL SAMPLE

M. Benczik¹, A. Kocsis², R. Koiss³, B. Járay⁴, I. Székely³, M. Nyíri², T. Takács¹

¹NEUMANN Diagnostics Ltd., Hungary, Cellcall Ltd., Hungary, Synlab Hungary Ltd. GenID Molecular Diagnostic Laboratory, Hungary (Hungary), ²NEUMANN Diagnostics Ltd., Hungary, Cellcall Ltd., Hungary (Hungary), ³Department of Obstetrics and Gynaecology, St. Stephan Hospital, Hungary (Hungary), ⁴2nd Department of Pathology, Semmelweis University, Hungary (Hungary)

Background / Objectives

Objectives. Several large population-based trials have demonstrated that tests detecting human papillomavirus (HPV) DNA of oncogenic types (hrHPV) are more effective in reducing cervical cancer than the cytology. The latest cervical cancer screening guidelines recommend hrHPV DNA testing as a primary screening test. The meta-analysis by Arbyn et al demonstrated that validated PCR-based tests detecting hrHPV DNA are as accurate on self-samples as on clinician-collected samples. Offering self-sampling kits for HPV testing is an opportunity to reach women who do not attend in the regular screening program, thereby increasing the effectiveness of the overall program.

Methods

Methods. Our aim is to validate the Qvintip (Aprovix) vaginal self-sampling device to the CONFIDENCE™ HPV test (Neumann Dx). CONFIDENCE™ HPV is a multiplex real-time PCR assay for HPV DNA detection. The test has a partial genotyping feature, it detects HPV16, 18, 31, 45 separately and other high risk types (HPV33, 35, 39, 52, 56, 58, 59, 66, 68) in group. The quality of the sample is guaranteed by the amplification of human control (factor V Leiden gene) and internal control. The internal control is an artificial DNA sequence added to the sample during DNA preparation.

In 2015 as a pilot study of a multicentric prospective clinical trial in Hungary, women aged between 18 and 65 years who attended cervical sampling at 4 clinical sites (among them outpatient and colposcopy clinics) were asked to self-collect a vaginal sample by using the Qvintip self-sampling kit, before the clinician obtained their cervical specimen in PreservCyt (Hologic). Currently we have collected 335 vaginal Qvintip and 335 cervical PreservCyt samples from these women.

Results

Results. The Qvintip collects the vaginal fluid by its collection head, which is then placed into a carrier tube provided by the kit and shipped to the laboratory. The preanalytical procedures for sample DNA preparation were adjusted to the vaginal sample type. We measured the cellularity of the Qvintip sample and diluted it with a buffer to a similar cellularity as the PreservCyt sample. We tested for a possible inhibitory effect of the vaginal sample type on the PCR reaction.

Conclusion

The CONFIDENCE™ HPV testing of the clinical samples is ongoing. We plan to specify the agreement of the CONFIDENCE™ HPV test on the Qvintip self-collected vaginal sample versus the clinician-collected cervical sample.

OC 12-14

COMPARATIVE STUDY OF THE DETERMINATION OF HPV TEST: SELF-SAMPLING VS URINE VS LIQUID MEDIUM CITOLOGY

J.M. Ramon Cajal, L. Ruiz Campo, M. Hernandez Aragon, C. Abad Rubio, L. Garcia Ponz, M. Garcia Calderon, F.J. Queipo Gutierrez

San Jorge General Hospital (Huesca) (Spain)

Background / Objectives

Chronic infection by the human papillomavirus (HPV) is the most important risk factor for developing cervical cancer. With the introduction of cervical cytology and population screening, the incidence and mortality from cervical cancer has been reduced. Isolated cytology has a sensitivity of 50%. Subsequently, techniques for detection of HPV DNA have been developed, and a sensitivity of more than 60-70% has been achieved. In any case, both techniques require a gynecological exam. In addition, they may not be possible in areas with low economic status. Therefore, other techniques have been developed for determining HPV in self-making or in urine collected by woman.

Methods

This is a prospective study on a sample of 100 patients from the Program of Screening for Cervical Cancer in our hospital area. As inclusion criteria in our study, the patients must have a positive determination of PCR of HPV DNA in liquid medium cytology using Cobas 4800® method, Roche Diagnostic Spain. This positive test may be accompanied by cytological alteration or not. These patients are informed to collect the samples (all of them first urine of the day and self-sampling) and derived to cervical pathology consultation. The self-sampling was taken using the device Qvintip® of the brand Aprovix.

Results

Finally 92 patients were included on the study (N=92). The average age was 40,2 years. 82,6% have had at least 3 couples in the last 3 years. 22,8% have not followed the correct cytological screening (7.6% had never previously made a cytology). About comfort in taking samples, 68.5% considered it more convenient and accessible than conventional cytology.

The results of the cytology were: 66,3% negatives, 23,9% ASC-US, 1,1% ASC-H, 6,5% L-SIL and 2,2% H-SIL. The types of HPV diagnosed were: 23,9% HPV 16, 2,2% HPV 18, 66,3% HPV no 16–18, 7,6% mixture. Biopsy was performed in 33.7% of cases, being positive in 61.3%.

Concordance of HPV in urine with cytology is 53,3% (44,4% for HPV 16, 25% for HPV 18 and 59% for other HPV). Concordance of HPV in self-sampling with cytology is higher, 73,9% (70% for HPV 16, 75% for HPV 18 and 75% for other HPV).

We analysed the 16 cases resulting in high-grade lesion on biopsy. If we compare the results for the urine to detect high-grade lesions sensitivity is 50%, specificity 44,7%, PPV 14,3% and NPV 82,9%. Regarding self-sampling: sensitivity 87,5%, specificity 28,9%, PPV 20,6% and NPV 91,7%.

Conclusion

The study highlights the higher concordance of self-sampling in high-grade lesions (87.5% of cases). It is a test with high sensitivity and NPV for high-grade lesions, which is what is intended, as it will be used in women who do not use conventional screening.

References

Mendez K, Romaguera J, Ortiz AP, López M, Steinau M, Unger ER. Urine-based human papillomavirus DNA testing as a screening tool for cervical cancer in high-risk women. *Int J Gynaecol Obstet.* 2014 Feb;124(2):151-5.

Bernal S, Palomares JC, Artura A, Parra M, Cabezas JL, Robles A, Martín Mazuelos E. Comparison of urine and cervical samples for detecting human papillomavirus (HPV) with the Cobas 4800 HPV test. *J Clin Virol.* 2014 Dec;61(4):548-52.

Sahasrabudde VV, Gravitt PE, Dunn ST, Brown D, Allen RA, Eby YJ et al. Comparison of human papillomavirus detections in urine, vulvar, and cervical samples from women attending a colposcopy clinic. *J Clin Microbiol.* 2014 Jan;52(1):187-92.

Gök M, Heideman DA, van Kemenade FJ, de Vries AL, Berkhof J, Rozendaal L et al. Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: Characteristics of the responders. *Eur J Cancer.* 2012 Aug;48(12):1799-808.

Arbyn M, Verdoordt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. *Lancet Oncol.* 2014 Feb;15(2):172-83.

OC 12-15

FAM19A4 methylation analysis in self-collected samples compared to physician-taken cervical scrapes for detection of cervical (pre)cancer in hrHPV-positive women

R. Luttmer¹, **L. De Strooper**¹, **M. Dijkstra**¹, **J. Berkhof**¹, **P. Snijders**¹, **R. Steenbergen**¹, **F. Van Kemenade**², **L. Rozendaal**¹, **T. Helmerhorst**², **R. Verheijen**³, **A. Ter Harmsel**⁴, **M. Van Baal**⁵, **P. Graziosi**⁶, **W. Quint**⁷, **J. Spruijt**¹, **D. Van Dijken**⁸, **D. Heideman**¹, **C. Meijer**¹

¹VU University Medical Center Amsterdam (Netherlands), ²Erasmus Medical Center Rotterdam (Netherlands), ³UMC Utrecht Cancer Center (Netherlands), ⁴Roosevelt Clinic Leiden (Netherlands), ⁵Flevo Hospital Almere (Netherlands), ⁶Sint Antonius Hospital Nieuwegein (Netherlands), ⁷DDL Diagnostic Laboratory Rijswijk (Netherlands), ⁸Onze Lieve Vrouwe Gasthuis Amsterdam (Netherlands)

Background / Objectives

Women who test high-risk human papillomavirus (hrHPV)-positive require triage to identify those with cervical high-grade intraepithelial neoplasia and cancer (\geq CIN3). *FAM19A4* methylation analysis, a promising triage marker which detects advanced CIN and cancer, is applicable to different sample types. However, studies comparing the performance of *FAM19A4* methylation analysis in hrHPV-positive self-samples and paired physician-taken scrapes are lacking.

Methods

We compared the performance of *FAM19A4* methylation analysis (with or without HPV16/18 genotyping) in self-collected lavages and paired physician-taken scrapes for \geq CIN3 detection in hrHPV-positive women (n=450, 18-66 years) visiting gynecologic outpatient clinics.

Results

In women \geq 30 years, \geq CIN3 sensitivity of *FAM19A4* methylation analysis was 78.4% in lavages and 88.2% in scrapes (ratio 0.89, CI:0.75-1.05). In women <30 years, \geq CIN3 sensitivities were 37.5% and 45.8%, respectively (ratio 0.82, CI:0.55-1.21). In both age groups, \geq CIN3 specificity of *FAM19A4* methylation analysis was significantly higher in lavages compared to scrapes (\geq 30 years:73.1% versus 63.2%; ratio 1.16, CI:1.03-1.30; <30 years:90.8% versus 82.2%; ratio 1.10, CI:1.02-1.20). In contrast to *FAM19A4* methylation analysis alone, the performance of combined

FAM19A4 methylation analysis and HPV16/18 genotyping did not differ significantly between lavages and scrapes.

Conclusion

FAM19A4 methylation analysis in hrHPV-positive self-collected lavages had a slightly lower sensitivity and a higher specificity for \geq CIN3 detection compared to *FAM19A4* methylation analysis in paired physician-taken scrapes. Combined *FAM19A4* methylation analysis and HPV16/18 genotyping revealed a similarly good clinical performance in both sample types. Therefore, this combination provides a feasible triage strategy for hrHPV-positive women, with the advantage of direct applicability on self-collected material.

OC 12-16

NOVEL DNA HYPERMETHYLATION MARKERS ARE FEASIBLE IN BOTH CERVICOVAGINAL LAVAGES AND CERVICAL SCRAPINGS

R.W. Van Leeuwen ¹, A.G.J. Van Der Zee ¹, J.J.H. Eijsink ², E. Schuurin
³, G.B.A. Wisman ³

¹Department of Gynaecologic Oncology, Cancer Research Centre Groningen, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands (Netherlands), ²Department of Obstetrics & Gynaecology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands (Netherlands), ³Department of Pathology, Cancer Research Centre Groningen, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands (Netherlands)

Background / Objectives

In 2017, the Dutch population-based screening program for the early detection of cervical cancer will be based on primary human papillomavirus (hrHPV) screening. However, the specificity of hrHPV testing, especially in a young screening population is relatively low, which may lead to unnecessary referrals to the gynaecologist, anxiety in the false-positive women, and higher costs for the health-care system. We have identified and validated a set of six new DNA methylation assays for early detection of cervical neoplasia in the hrHPV-positive subpopulation. In the new primary HPV screening program, it is expected that a significant number of women who did not participate in the current cervical cancer screening program will attend upon receiving a self-collection device. Here we assessed whether DNA hypermethylation analysis is feasible in self-collected specimens.

Methods

Using quantitative methylation-specific PCR (QMSP) six markers (ANKRD18CP, GFRA1, KCNIP4, SOX1, SOX14, ZSCAN1) were analysed on DNA extracted from 41 sample pairs (CIN0/1 n=7, CIN2 or worse (CIN2+) n=34) that were simultaneously collected by Delphi screener and routine collection. Concordance in test classification was determined with Cohen's kappa and Spearman's rho. Comparisons between receiver-operator characteristics (ROC) were analysed using DeLong's test. Data on four additional methylation markers (C13orf18, EPB41L3, JAM3 and TERT), liquid-based cytology and Hybrid Capture II hrHPV testing was also available.

Results

Between sampling types, all methylation markers showed significant concordance (each $\kappa \geq .50$, each $p' < .01$) and a significant correlation (each $\rho \geq .74$, each $p' < 10^{-6}$). No differences were observed between paired ROCs (each $p' > .1$). Of all tests performed, ZSCAN1 and EPB41L3 showed the highest diagnostic potential to detect CIN2+ with an area under the ROC (AUC) of .97 and .91 in clinician-collected smears and .93 and .84 in lavages, respectively.

Conclusion

Analysis of these methylation markers is feasible in cervicovaginal lavages. This research further encourages the introduction of methylation analysis to detect cervical neoplasia in the new population-based screening program irrespective of sampling type.

OC 12-17

Acceptability of HPV testing using a self-sampling device in non-attendees of municipal cervical cancer screening in Japan

M. Ito¹, H. Konishi², O. Iwanari³, Y. Ohashi⁴, Y. Matsuyama⁴

¹Institute for Future Engineering, the University of Tokyo (Japan), ²Japan Cancer Society (Japan), ³Shimane Prefectural Central Hospital (Japan), ⁴the University of Tokyo (Japan)

Background / Objectives

The Japanese government recommends that adult females receive cervical cancer screening by Pap smear test every other year. However, according to the National Livelihood Survey in 2013, the coverage is only 32.7%. Recently the number of young Japanese women in their late 20s and 30s who get cervical cancer has been increasing. Izumo city in the western part of Japan started HPV co-testing for cervical cancer screening in 2007, much earlier than other municipalities. Adopting HPV co-testing showed successful results and there had been only few patients who needed extended hysterectomy. However, about half of the adult females did not receive the cervical cancer screening in the past 5 years, especially 60% of those in their late 20s and 30s. All cases with extended hysterectomy were from the non-attendees of screening in the past. Our ultimate aim is to improve the coverage of cervical cancer screening. In this study, we investigate if the self-sampling HPV test is an effective way to approach non-attendees and let them receive cervical cancer screening.

Methods

The target candidates of this study were women living in Izumo city, who were 25-year old to 45-years old, and who did not attend Izumo city's cervical cancer screening in the past 5 years. In July 2015, we sent letters to let them know that they can receive free HPV tests at home using a self-sampling device. If they were willing, we sent them the HPV self-sampling device with a questionnaire on cervical cancer screening. In October we sent reminder letters to those who did not send back the self-collected sample and answered questionnaires. We used Evalyn Brush as the HPV self-collection device, and Hybrid Capture 2 (HC2) as the assay for high risk HPV DNA

Results

Number of the candidate was 12,546. 2,120(16.9%) of them received the self-sampling HPV test, although 2806 of them wanted to receive self-sampling HPV test. 152 of attendee (7.2%) were HPV positive. 106 women of HPV positive received the

Pap smear test by January in 2016. The result of Pap test was following; NILM(63), ASC-US(10), LSIL(24), HSIL(7), AIS(1), Improper sample(1). Almost all of participants of self-sampling HPV test answered that the result of self-sampling HPV test, especially HPV positive, could be good motivation to receive municipal cervical cancer screening using Pap test. 91.5% of participants thought that they would receive screening regularly if self-sampling test is available.

Conclusion

Although further analysis and research are needed, HPV test using self-sampling device is very useful and acceptable to non-attendees of the present cervical cancer screening with Pap smear test. It has the potential to improve the coverage of screening.

OC 12-18

ACCEPTABILITY OF SELF-SAMPLING FOR CERVICAL CANCER SCREENING BY HEALTH CARE PROVIDERS IN THE ACCESSING PROGRAM

A.L. Behnke¹, **C.M. Wormenor**², **P. Dunyo**², **A. Krings**¹, **A.M. Kaufmann**¹, **J.E. Amuah**³

¹Department of Gynecology (CBF), Charité - Universitätsmedizin Berlin (Germany), ²Catholic Hospital Battor (Ghana), ³School of Epidemiology, Public Health and Preventive Medicine, Faculty of Medicine, University of Ottawa (Canada)

Background / Objectives

Self-sampling is a reliable and acceptable screening approach in hard-to-reach women and low-resource settings (1). ACCESSING (Adequate Cervical cancer Capacity building, Education and Screening by new Scientific Instruments in Ghana) is a feasibility study for self-sampling in rural communities of the North Tongu district in Ghana. In order to acquaint female health care workers with the sampling method, female employees were invited to self-collect a cervicovaginal lavage (DELPHI Screener™) and were interviewed to explore acceptability and their potential role as screening advocates.

Methods

A mixed methods approach was used to evaluate the acceptability of self-sampling among female health care providers working in a district hospital. Ethical clearance and written informed consent was obtained for sampling and interviews. 52 staff members who had self-collected a sample filled in questionnaires. A qualitative study obtained in-depth information on opinion, preference and implications of participating in the self-sampling workplace program. Ten participants were purposively sampled and interviewed. Interviews were analyzed according to qualitative content analysis guidelines.

Results

The quantitative analysis of the questionnaires (n=52, average age: 36 years, SD 10.6, range 23 to 59) showed that ~96% (50/52) took the sample themselves and ~2% (1/52) had the sample taken by a fellow health worker at the clinic (1/52 n.a.). Of the ones that took the sample themselves 100% (50/50) found it "Easy" (9) or "Very Easy" (41). ~92% (46/50) felt "Very Comfortable" and ~8% "Comfortable". ~83%

(43/52) indicated that they would get checked more often if the Delphi Screener works as well as going to see a doctor at a clinic to get sampled. ~ 98% (51/52) indicated they would prefer self-sampling if their risk is as reliably determined as by physician-directed cytobrush sampling.

All interview participants (n=10, average age: 40.9 years, SD 10.8, range 28 to 59) indicated that they appreciate the program and recommend to their patients and/or family members and neighbours to get screened for cervical cancer. Common reasons for preferring self-sampling were less (anticipated) pain compared to speculum examination and more privacy.

Conclusion

Self-sampling for cervical cancer screening is highly acceptable to female healthcare providers who took a sample themselves. Setting up a workplace program that entails the possibility of self-sampling can help to ensure effective cervical cancer screening and at the same time creates awareness among healthcare providers to educate their patients as well as families and neighbours on cervical cancer and advocate for women's participation in screening.

References

((1) Arbyn M et al., Lancet Oncol. 2014 ;15(2):172-83)

OC 12-19

MODERN TECHNOLOGY-BASED COMMUNICATION PLATFORMS ARE WELL ACCEPTED FOR SCREENING PARTICIPATION OF NON-ATTENDERS THROUGH SELF-SAMPLING

J.U.H. Lam¹, M. Rebolj², D.M. Ejegod¹, H. Pedersen¹, C. Rygaard¹, E. Lynge³, E.H. Christensen⁴, S.K. Kjaer⁴, J. Bonde⁵

¹Department of Pathology, University Hospital Copenhagen (Denmark),

²Clinical Research Centre, Copenhagen University Hospital Hvidovre (Denmark), ³Department of Public Health, University of Copenhagen (Denmark),

⁴Unit of Virus, Lifestyle and Genes, Danish Cancer Society Research Center

(Denmark), ⁵Department of Pathology, University Hospital Copenhagen; Clinical Research Centre, Copenhagen University Hospital Hvidovre (Denmark)

Background / Objectives

Fifty percent of newly diagnosed cervical cancers are found among screening non-attenders. A quarter of Danish women do not attend screening; hence, the Copenhagen self-sampling initiative aimed to increase the screening coverage by offering non-attenders a Human Papillomavirus (HPV)-based self-sampling test. Women had to opt in by using conventional mail based response or one of the available electronic response methods including QR code mobile/web directed webpage, and e-mail. To understand the extent of acceptability of electronic communication platforms for opt-in strategies, we here describe the non-attenders by their screening history, participation rate and the usage of the different response methods.

Methods

In total, 23,362 non-attenders from the Capital Region were invited for participation. These women were not screened for at least one screening round, as ascertained through the national Pathology Data Bank. We determined the women's responses in 7 months after the invitation. Women could order a self-sampling test via traditional response methods (regular mail, phone call) or via a webpage or email. The latter could be accessed directly or by use of supplied QR-code. Women were divided into two groups in line with their screening history: long-term unscreened women (no cytology sample registered in the last 10 or more years) and intermittently screened women (at least one cytology sample registered within the last 10 years but not within the last screening round). Pearson's χ^2 -test was used to compare the differences in the responses and in participation by screening history and by response methods.

Results

Of all invited women, 32% agreed to participate, and 20% returned the self-sampling test. The intermittently screened women were statistically more likely to return the test than the long-term unscreened women (36% vs. 24%, $p < 0.001$). In total, 68% of all responses were received via regular mail or phone, and 32% via the webpage. Web-users were statistically more likely to opt in than regular mail users (98% vs 79%, $p < 0.001$), but only slightly more likely to return the test (66% vs 63%, $p = 0.01$).

Conclusion

Intermittently screened women were more likely to participate in self-sampling than long-time unscreened women. Other methods may be considered to reach out to the latter women, for instance a more targeted text in the invitation letter. Furthermore, women using modern technology platforms were more likely to participate than regular mail-users. Because of the lower cost of setting up technology-based systems than large scale use of regular mail service, electronic platforms should be considered in self-sampling using an opt-in strategy.

OC 13-01

BRAZILIAN PUBLIC HPV VACCINATION PROGRAM: FIRST TWO YEARS OF EXPERIENCE

L. Resende

HRAN/SESDF (Brazil)

Background / Objectives

INTRODUCTION: Cervical cancer is the fourth cause of death in women worldwide. In Brazil, 17,540 women were diagnosed in 2012 with the disease. The screening program for cervical cancer is not organized and cytology (Pap smear) is performed for women aged 25-64 years. The use of quadrivalent HPV vaccine started on 2013 and the first city to adopt the program was Brasilia – DF. **OBJECTIVE:** To provide background data for strengthening cervical cancer prevention in Brazil. Describe the importance of a large populational public vaccine program for prevent cervical cancer in the next decades.

Methods

METHODS: Objective analysis of the record of women who received the HPV quadrivalent vaccine in Brasilia - DF in the years 2013-2014. The data was provided for the District Board of Health.

Results

RESULTS: Brazilian HPV vaccination program used quadrivalent vaccine and enroll women aged 9-13 years. It was performed at public schools with 3 doses (0-2-6 months), it was funded by the Ministry of Health with no extra cost to the population. 62.854 women were eligible for the vaccine in Brasilia and the coverage of first dose was 94%; 94,04% second dose and 88,86% the last dose.

Conclusion

CONCLUSION: Our data indicate that the vaccination of young women may be the right strategy for prevention of pre-invasive and invasive squamous lesions due to reduce the prevalence and persistent HPV infection. However, it needs an organized vaccination program, with the appropriate target population coverage and to maintain a high rate of adherence of these women.

OC 13-02

EFFECTIVENESS OF THE QUADRIVALENT HUMAN PAPILOMAVIRUS VACCINE AGAINST ANOGENITAL WARTS IN MANITOBA, CANADA: A POPULATION-BASED STUDY

K. Willows, S. Bozat-Emre, E. Kliewer, S. Mahmud

**Department of Community Health Sciences, Faculty of Health Science,
University of Manitoba (Canada)**

Background / Objectives

The quadrivalent human papillomavirus vaccine (QHPV) became available in Manitoba in 2006, and was introduced into the publicly-funded school-based program in 2008. Anogenital warts (AGWs) are one of the earliest clinical outcomes of HPV infection. They tend to occur at a younger age than other HPV-related disease, and can therefore provide an early indication of the success of school-based vaccination programs. However, to date, few studies have assessed the effectiveness of these programs in preventing AGWs using population-based, individual-level data. We used a historical matched cohort study to assess the effectiveness of the QHPV program in Manitoba, in reducing the incidence of medically-attended AGWs and, whether effectiveness depends on age at vaccination, and evidence of prior sexual activity.

Methods

Using Manitoba's population-based vaccine registry, we identified females > 9 years old who received QHPV between September 2006 and March 2013 (n=31,464). They were matched, with replacement, on age and area of residence to three unvaccinated females (n=94,327). Information on incident AGWs was obtained from hospital, physician and drug prescription databases using validated algorithms. Evidence of prior sexual activity was determined using a composite of codes for pregnancy, sexually transmitted infection, or contraceptive drug use. We used Cox regression models, stratified to account for matching, to determine hazard ratios for AGW among the vaccinated, compared to the unvaccinated.

Results

We identified 500 cases of incident AGWs. QHPV was associated with 40% reduction in AGW risk (HR 0.6, 95% CI 0.4-0.8) among females vaccinated before they turned 18. Among Females vaccinated at >18 years of age, QHPV was associated with increased AGW risk, especially among those who were sexually active (HR 2.8, 95% CI 2.1-3.7), likely because of increased QHPV use among high-risk women. Adjustment for socioeconomic and medical history covariates did not alter these estimates.

Conclusion

In order to optimize our current publicly-funded QHPV vaccination program in Manitoba, further efforts should be targeted at increasing vaccine uptake in young adolescents, prior to the initiation of sexual activity.

OC 13-03

EFFECTIVENESS, IMMUNOGENICITY, AND SAFETY OF GARDASIL™ IN PRE-ADOLESCENTS AND ADOLESCENTS – 10 YEARS OF FOLLOW-UP

R. Das¹, A. Saah¹, O.E. Iversen²

¹Merck & Co., Inc. (United States of America), ²Norwegian Institute (Norway)

Background / Objectives

Quadrivalent HPV vaccine has previously been shown to be effective, immunogenic, and safe in pre-adolescents and adolescents aged 9-15, through 8 years after vaccination. We describe final 10-year data for the long-term follow-up (LTFU) study of GARDASIL™ in this population.

Methods

In the base study of V501-Protocol 018, 1661 sexually naïve boys and girls were assigned to GARDASIL or placebo at day 1, months 2 and 6. At the end of the base study (month 30), the placebo group received GARDASIL™. Those vaccinated with GARDASIL™ in the base study are the early vaccination group (EVG). Those vaccinated with GARDASIL™ thereafter are the catch-up vaccination group (CVG). As this LTFU study does not have a placebo arm, effectiveness was estimated by calculating the incidence rate of the primary endpoints (HPV6/11/16/18 related disease or persistent infection) and comparing with rates from previous studies in young adults (aged 16-26).

Results

A total of 1245 subjects (821 in the EVG and 424 in the CVG) had visits in the LTFU study. The median follow-up time was 9.9 years in EVG and 7.4 years in the CVG. No cases of HPV 6/11/16/18-related disease were observed. Ten subjects were detected to have persistent infection of ≥6month duration with vaccine-type HPV (females: 0.3/100 person-years at risk in the EVG and CVG, males: 0.6/100 person-years at risk in the EVG and 0.4/100 person-years at risk in the CVG). Infection persisted for ≥12 months in only 2 of these subjects. Incidence of HPV 6/11/16/18 persistent infection in female and male placebo recipients from previous studies were 6/100 person-years at risk and 4/100 person years at risk respectively. For each of HPV types 6, 11 and 16, 89%-96% remained seropositive through 10-years post-vaccination. Lower anti-HPV 18 responses were seen over time, consistent with observations in other studies of GARDASIL™ but no cases of

persistent infection due to HPV type 18 were observed. No serious adverse events were reported between 8 and 10 years.

Conclusion

No breakthrough cases of cervical/genital disease related to HPV types 6, 11, 16, and 18 were observed among preadolescents and adolescents vaccinated with GARDASIL™ during 10 years of follow-up. Although 10 cases of persistent infection were detected, a majority (8/10) were of <12 months duration. Anti-HPV 6, 11, 16, and 18 antibody responses post-vaccination persisted over time. Additionally, the safety profile of GARDASIL™ during the LTFU period remained favorable.

OC 13-04

IMPACT AND EFFECTIVENESS OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE: TEN YEARS OF REAL WORLD EXPERIENCE

S. Garland¹, **S. Kjaer**², **N. Munoz**³, **S. Block**⁴, **D. Brown**⁵, **M. Dinubile**⁶, **B. Lindsay**⁶, **B. Kuter**⁶, **G. Perez Amaya**⁶, **G. Dominiak-Felden**⁷, **A. Saah**⁶, **R. Drury**⁷, **R. Das**⁸, **C. Velicer**⁸

¹The Royal Women's Hospital (Australia), ²University of Copenhagen (Denmark), ³Colombian National Institute of Cancer (Colombia), ⁴Kentucky Pediatric and Adult Research (United States of America), ⁵Indiana University School of Medicine (United States of America), ⁶Merck & Co., Inc. (United States of America), ⁷Sanofi Pasteur MSD (France), ⁸Merck & Co, Inc. (United States of America)

Background / Objectives

Prophylactic HPV vaccine programs primarily targeting adolescent populations prior to sexual debut constitute a major worldwide public-health initiative. We assessed the global real-world effect of the quadrivalent HPV (4vHPV) vaccine containing HPV types 6/11/16/18 over its first decade of use.

Methods

We systematically searched PubMed and Embase for peer-reviewed articles published in any language between January 2007 and November 2015 to capture observational studies (including national registries) evaluating the impact or effectiveness of 4vHPV vaccination against HPV infection, anogenital warts, low-grade cervical cytological and histological abnormalities (ASCUS, LSIL, CIN1), and/or high-grade cervical lesions (HSIL, ASC-H, CIN2, CIN3, adenocarcinoma in situ (AIS), cervical cancer). We limited the search to articles reporting on the 4vHPV vaccine.

Results

A rapid and dramatic reduction in the prevalence of HPV 6/11/16/18 infection (~40% to 80%) and genital warts (up to 90%) after introduction of 4vHPV vaccination programs was demonstrated in young women in Australia, Europe, North America, and New Zealand. In subsequent years, as successive birth cohorts began cervical screening, reductions in cervical lesions started to become apparent (e.g., ~10% to 35% reduction in low-grade abnormalities, ~25% to 80% reduction in high-grade abnormalities). In Australia and Denmark where programs have achieved high and

timely vaccination coverage and include catch-up vaccination, respective reductions as high as 47% and 80% in CIN3 lesions have been reported in the youngest cohorts vaccinated shortly after program implementation. Overall, while 4vHPV vaccine programs have been successful in reducing HPV 6/11/16/18 infection and HPV-related disease, the actual estimates of impact and effectiveness vary regionally based on age at vaccination, vaccination coverage, the number and timing of vaccine doses, length of follow-up, cervical cancer screening recommendations and practice, statistical power, completeness and accuracy of data sources, and analytic methods.

Conclusion

Over the last decade, the impact of HPV vaccines in real-world settings has become increasingly evident, especially where there is broad coverage of the target population. Despite high vaccine effectiveness, the full public-health potential of HPV vaccination is unfortunately far from being realized. Many preventable HPV-related diseases continue to present major public health challenges for both developing and developed nations, underscoring the need for wide-reaching HPV vaccination programs with high population coverage prior to sexual debut.

OC 13-05

COST-EFFECTIVENESS EVALUATION OF THE QUADRIVALENT HPV VACCINE IN SOUTH KOREA USING A DYNAMIC TRANSMISSION MODEL

S. Kim¹, M. Jo², C. Park³, S. Kang³, M. Pillsbury⁴, A. Kulkarni⁴

¹Dankook University (Korea, republic of), ²University of Ulsan (Korea, republic of), ³Merck Sharp & Dohme, Corp. (Korea, republic of), ⁴Merck & Co., Inc. (United States of America)

Background / Objectives

Human papillomavirus (HPV) infection is associated with cervical cancer and with other anogenital and head and neck cancers and anogenital warts. Two HPV vaccines are currently licensed in the South Korea, the bivalent HPV vaccine which targets HPV types 16 and 18 (which are associated with 70–80% of cervical cancers), and the quadrivalent HPV vaccine, which also targets HPV types 6 and 11 (associated with 85–95% of cases of anogenital warts) in addition to HPV type 16 and 18. WHO recommended that routine HPV vaccination should be included in national immunization program. In Korea, public vaccination program are not implemented yet and there have been no cost-effectiveness analyses for quadrivalent HPV vaccine compared with bivalent HPV vaccine.

To examine cost effectiveness of quadrivalent human papillomavirus (HPV) vaccine compared with bivalent vaccine and no vaccination (screening only) in Korea.

Methods

We adapted previously published dynamic transmission mode and calibrated it to the Korean context simulating the natural history of cervical cancer and genital warts in Korea. We assumed that the vaccination program would be combined with current cervical cancer screening in Korean. Vaccination strategy is HPV vaccination of girls at the age of 12, who receive two doses of HPV vaccine (80% coverage). We assumed that the relative effectiveness of two dose of vaccine was the same as three doses and the duration of protection of HPV vaccination to be life-long for both vaccines. We did not consider other HPV type except HPV type 6, 11, 16 and 18 in this model. The costs of vaccinations were assumed based on 70% of estimated market prices; the cost of quadrivalent vaccination was assumed as KRW 108,000 and the cost of bivalent vaccination was assumed as KRW 81,000.

Results

In the base case analysis, the incremental cost-effectiveness ratio (ICER) of the quadrivalent and bivalent vaccines compared with current screening only strategy were KRW 13,727,353 (\$ 11,439, \$1 = 1,200KRW) and KRW 23,999,474 (\$20,000) per QALY-gained, respectively. The ICER of quadrivalent vaccine versus bivalent vaccine was KRW 4,677,273 (\$ 3,898).

Conclusion

Vaccinating 12- year- old girls against HPV with the quadrivalent HPV vaccine is estimated to be cost-effective over the bivalent vaccines in the Korean setting considering the criteria for threshold of ICER in Korea ranges from KRW 20,000,000 to 30,000,000 (\$16,000 to \$25,000). In addition, ICER of the quadrivalent HPV vaccine was lower than ICER of the bivalent HPV Vaccine when compared with current screening only strategy.

OC 13-06

PUBLIC HEALTH BENEFITS OF ROUTINE HUMAN PAPILLOMAVIRUS VACCINATION FOR ADULTS IN THE NETHERLANDS: A MATHEMATICAL MODELING STUDY

S. Matthijsse, J. Hontelez, S. Naber, K. Rozemeijer, R. Bakker, M. Van Ballegooijen, J. Van Rosmalen, S. De Vlas

Erasmus MC (Netherlands)

Background / Objectives

In most countries, human papillomavirus (HPV) vaccination uptake in girls is relatively low, and, together with the limited target age range, this has led to HPV transmission control not reaching its full potential. The multinational VIVIANE study showed that the bivalent HPV vaccine is efficacious in adult women. Integrating adult HPV vaccination into existing public health programs could therefore be an effective strategy to improve HPV and cervical cancer prevention. The objective of our study was to estimate the impact of adding adult HPV vaccination to the current HPV vaccination program in the Netherlands.

Methods

We used the established STDSIM microsimulation model to evaluate the following adult vaccination strategies for only adult women or both men and women, in addition to the current girls-only vaccination program: a one-time mass campaign for the 24-45 year age group, vaccination at the first cervical cancer screening visit for 30-year old women, vaccination at sexual health clinics for the 15-29 year age group, and combinations of these strategies. Outcome measures were incremental incidence reductions of HPV-16 and HPV-18 and incremental number needed to vaccinate (NNV) to prevent an HPV-16/18 infection, both compared to the current Dutch vaccination program.

Results

The estimated impact of extending vaccination to adult women is modest, with largest incremental HPV incidence reductions occurring when offering vaccination both at the first cervical cancer screening visit and at sexual health clinics (i.e. about 20% lower after 50 years for both HPV-16 and HPV-18). The largest incidence reduction was achieved by adding male vaccination at sexual health clinics to this strategy

(63% for HPV-16 and 84% for HPV-18). For this strategy, the incremental NNV to prevent one infection in women is 5.48, compared to 0.90 for the current vaccination program.

Conclusion

Extending HPV vaccination to adult women and men would substantially reduce HPV incidence in the Netherlands. Since existing infrastructures can be used, HPV vaccination for adults, especially at the first cervical cancer screening visit (for women) and at sexual health clinics (both sexes), would be an important and highly efficient policy measure to further improve HPV prevention and subsequently avert cervical and other HPV-related cancers, both in men and women.

OC 13-07

PUBLIC HEALTH IMPACT OF A NINE-VALENT HPV VACCINATION PROGRAM FOR FEMALES IN HUNGARY USING A DYNAMIC TRANSMISSION MODEL

A. Kulkarni¹, A. Pavelyev², J. Kalmar³, L. Nagy³, M. Pillsbury¹, Z. Szentirmay⁴, M. Kasler⁴

¹Merck & Co., Inc. (United States of America), ²HCL America, Inc. (United States of America), ³Merck Sharp & Dohme, Corp. (Hungary), ⁴National Institute of Oncology (Hungary)

Background / Objectives

To assess public health impact of the introduction of nine-valent human papillomavirus (HPV) vaccine program for females in Hungary.

Methods

A previously published HPV dynamic transmission model was adapted and calibrated to the Hungarian setting. The model simulated the natural history of cervical cancer, anogenital caners, and genital warts in Hungary. It was assumed that the vaccination program would be combined with current cervical cancer screening in Hungary. The model assumed that 85% of females aged 13-14 years would receive two doses of the nanovalent HPV vaccine. The relative effectiveness of two dose of the nine-valent HPV vaccine was assumed to be the same as three doses and the duration of protection of the nine-valent HPV vaccine to be life-long. Impact of other HPV types except the nine HPV types in the nine-valent HPV vaccine was not considered. The nine-valent female HPV vaccination program (combined with cervical cancer screening) was compared with no HPV immunization (cervical cancer screening only). The time horizon for the model was 100 years. Costs were discounted at 3.7%.

Results

For the comparison with no HPV immunization, over a 100 years, the introduction of the nine-valent vaccination program in females showed cumulative percent reduction in the incidence of 6/11/16/18/31/33/45/52/58 HPV related cervical cancer by 52%, CIN1 by 69%, and CIN2/3 by 66%, anal cancer in females and males by 41% and 34% respectively, cervical cancer related deaths by 49%, anal cancer related death

by 40% in females and 32% in males, HPV 6/11 related genital warts in females and males by 78% and 71% respectively. By the end of the 100 year timeframe of the model, the nine-valent HPV vaccine reduced the monitored incidence rates and related death cases for cervical and anal cancers to near zero. Over a 100 years, the nine-valent HPV vaccine would reduce cumulative cost of 6/11/16/18/31/33/45/52/58 HPV related cervical cancer by 18%, CIN1 by 34%, CIN2/3 by 31%, anal cancer in females and males by 12% and 10% respectively, and HPV 6/11 related genital warts in females and males by 51% and 44% respectively.

Conclusion

The introduction of the nine-valent HPV vaccination program for females in Hungary will significantly reduce cervical and anal cancer cases and associated costs. The burden and costs related to various 6/11/16/18/31/33/45/52/58 HPV-related conditions could be substantially reduced by the introduction of a nine-valent HPV vaccination program for females in Hungary.

OC 13-08

WOLVES-STUDY – IMPACT OF HPV VACCINATION IN WOLFSBURG (GERMANY)

A. Luyten¹, S. Strehlke¹, V. Weiß¹, T. Iftner², A. Iftner², K.U. Petry¹

¹Department for Gynecology, Obstetrics and Gynecologic oncology, Wolfsburg (Germany), ²Dept. of Med. Virology and Epidemiol. of Viral Diseases, Tuebingen (Germany)

Background / Objectives

In Germany data on the epidemiology of HPV infections and especially on the impact of HPV vaccination are rare. WOLVES (Wolfsburg HPV epidemiology study) is the first population based surveillance study in Germany to measure the impact of HPV vaccination and the burden of HPV related diseases in a two different birth cohorts.

Methods

All women born 1993/84 and 1988/89 who were registered in the city of Wolfsburg were invited by letter. Participants filled in a standardized questionnaire and underwent pelvic examination with Pap smear and HPV testing (HC2-LR and HR). HPV genotyping (LiPA) was done in all HC2 positive samples. Women with genital warts (GW) or atypical smears were transferred to colposcopy.

Results

53.6% (n=148) of the 1993/94 and 18.7% (n=221) of the 1988/89 target populations were vaccinated until October 2015. The overall HPV prevalence (HC2 HR & LR) was 25.9%. Between the two age groups significant differences were observed (22.4% vs. 26.6%). In the 1988/89 cohort 2.7% (n=6) were tested positive for HPV 6/11/16/18 in vaccinated women vs. 11.2% (n=108) in not vaccinated women and 0% vs. 4 (3.1%) in the 1993/94 cohort. In women with initially negative HPV result (n=848) 182 were tested HPV positive once in 5 year follow up with biopsy proven CIN2+ (0.36%). In HPV positive women (n=308) the risk for CIN2+ into 5 years was 11.7% (n=36).

Conclusion

Although vaccination rates were relatively low an impact of HPV vaccination is visible.

OC 13-09

PUBLIC HEALTH IMPACT OF A NINE-VALENT HPV VACCINATION PROGRAM FOR FEMALES AND MALES IN HUNGARY USING A HPV TRANSMISSION DYNAMIC MODEL

A. Kulkarni¹, A. Pavelyev², J. Kalmar³, L. Nagy³, M. Pillsbury¹, Z. Szentirmay⁴, M. Kasler⁴

¹Merck & Co., Inc. (United States of America), ²HCL America, Inc. (United States of America), ³Merck Sharp & Dohme, Corp. (Hungary), ⁴National Institute of Oncology (Hungary)

Background / Objectives

To assess public health impact of the introduction of nine-valent human papillomavirus (HPV) vaccine program for females and males in Hungary.

Methods

A previously published HPV dynamic transmission model was adapted and calibrated for Hungary. The natural history of cervical cancer, anogenital cancers, and genital warts in Hungary was simulated using the model. It was assumed that the vaccination program would be combined with current cervical cancer screening in Hungary. The model assumed that 85% of females aged 13-14 years and 50% of the boys aged 13-14 years would receive two doses of the nonavalent HPV vaccine. The relative effectiveness of two dose of the nine-valent HPV vaccine was assumed to be the same as three doses. The duration of protection of the nine-valent HPV vaccine was assumed to be life-long. Impact of only HPV types 6/11/16/18/31/33/45/52/58 were considered in the model. The nine-valent female and male HPV vaccination program (combined with cervical cancer screening) was compared with no HPV immunization (cervical cancer screening only). The time horizon for the model was 100 years. Costs were discounted at 3.7%.

Results

For the comparison with no immunization, over a 100 years, the introduction of the nine-valent vaccine program in females and males showed cumulative reduction in the incidence of 6/11/16/18/31/33/45/52/58 HPV related cervical cancer by 54%, CIN1 by 70%, and CIN2/3 by 67%, anal cancer in females and males by 43% and 41% respectively, cervical cancer related deaths by 50%, anal cancer related death

by 41% in females and 39% in males, HPV 6/11 related genital warts in females and males by 82% and 81% respectively. By the end of the 100 year timeframe of the model, the nine-valent HPV vaccine reduced the monitored incidence rates and related death cases for cervical and anal cancers to near zero. Over a 100 years, the nine-valent HPV vaccine would reduce cumulative cost of 6/11/16/18/31/33/45/52/58 HPV related cervical cancer by 19%, CIN1 by 35%, CIN2/3 by 31%, anal cancer in females and males by 13% and 12% respectively, and HPV 6/11 related genital warts in females and males by 54% and 52% respectively.

Conclusion

The burden and costs related to various 6/11/16/18/31/33/45/52/58 HPV-related conditions, especially cervical and anal cancers, could be substantially reduced by the introduction of a nine-valent HPV vaccination program for females and males in Hungary.

OC 13-10

LONG-TERM SAFETY OF THE HPV-16/18 ASO4-ADJUVANTED VACCINE

K. D'hauwers¹, **W. Tjalma**²

¹Radboud university medical center, Urology, Nijmegen (Netherlands),

²University Hospital Antwerp, Oncological Gynecology, Antwerp (Belgium)

Background / Objectives

HPV vaccines would only be well-marketed products, inducing unacceptable sexual behavior and causing serious adverse effects. Gardasil® contains aluminum salts and Cervarix® the ASO5-adjuvant. To stimulate the immune response immunoenhancers are included in the adjuvant formulation. We want to give an overview of long term safety of the ASO4-adjuvanted vaccine.

Methods

Literature.

Results

In the timeframe around immunization implementation, the number of reported concurrent autoimmune diseases will increase. Therefore, their prevalence needs to be monitored in advance. New onset of chronic diseases (NOCDs, mainly asthma, urticaria and hypersensitivity): 1.7% in the HPV16/18 group and 1.7% in the control group. The frequency did not differ between the groups during all follow-up periods. [1] New onset of autoimmune diseases (NOADs, mainly thyroid-related): 0.4% in the HPV16/18 group and 0.3% in the control group. The frequency did not differ between the groups during all follow-up periods.[1] Pregnancy: The percentage of spontaneous abortion in those exposed to vaccination within 60 day before pregnancy was 15.1% vs. 9.5% in a control group [2]. In an animal study, where the vaccine was administered to parental female rats, there was no negative effect on their clinical condition, bodyweight, food consumption. Their pregnancy-rates and embryo fetal growth were unaffected. The survival and development of the offspring was equal to the control animals (injection with saline). Transfer of antibodies to fetuses and pups occurred in utero and during lactation, respectively . The concentration of antibodies in the rat fetuses was around 20% of that found in the vaccinated dams. [3] Deaths: In 2014, on 57 580 subjects, 63 deaths were administered: 25 in recipients, 20 in controls and 18 in blinded groups. Common causes were suicide, malignancy, infections and road accidents. [4] Solicited symptoms: Injection site symptoms and some general symptoms were more common

in the vaccine group than in control groups [2] Most common unsolicited symptoms were events to be expected in the study population.

Conclusion

The HPV-16/18 AS04-adjuvanted vaccine is safe. Point of attention might be not to get pregnant shorter than 60 days after administration.

References

- [1] Safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for cervical cancer prevention: a pooled analysis of 11 clinical trials. Descamps D, et al. *Hum Vaccin* 2009. [2] Strategies for continuous evaluation of the benefit-risk profile of HPV-16/18-AS04-adjuvanted vaccine. Angelo MG, et al. *Expert Rev Vaccines* 2014. [3] Evaluation of the intramuscular administration of Cervarix™ vaccine on fertility, pre- and post-natal development in rats. Segal L, et al. *Reprod Toxicol* 2011 [4] Pooled analysis of large and long-term safety data from the human papillomavirus-16/18-AS04-adjuvanted vaccine clinical trial programme. Angelo MG, et al. *Pharmacoepidemiol Drug Saf* 2014

OC 13-11

SAFETY AND IMMUNOGENICITY OF THE HPV-16/18 AS04-ADJUVANTED VACCINE IN ADOLESCENTS: FINAL ANALYSIS OF A LARGE COMMUNITY-RANDOMIZED TRIAL IN FINLAND

D. Bi

GSK Vaccines, Clinical Research and Development, Wavre, Belgium (Belgium)

Background / Objectives

To evaluate safety and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in a large prospective, community-based controlled study (HPV-040, NCT00534638).

Methods

In this phase III/IV, cluster-randomized, partially blind trial, adolescents aged 12–15 years (y) born 1992–1995, from 33 communities in Finland, received HPV-16/18 (12399 girls, 2438 boys) or hepatitis B virus (HBV) vaccine (8119 girls, 9219 boys), at months (m) 0-1-6. Subjects were followed-up until they reached ~18.5y of age. Active safety surveillance was performed until m12 in a subset of boys. Passive safety surveillance was performed by linkage of the Registry of Vaccinated Individuals (total vaccinated cohort: 32175 subjects) to a national hospital patient register (HILMO; occurrence of new onset of autoimmune disorders [NOADs] and pregnancy-related outcomes) and to the Birth Registry (pregnancy-related outcomes). Spontaneous reporting of related serious adverse events (SAEs) and pregnancy outcomes was recorded throughout the study period. Blood samples were collected at m0, m7 and at age ~18.5y; anti-HPV-16/18 antibody responses were assessed by ELISA (immunogenicity subset: 764 girls and 225 boys).

Results

Active safety surveillance results were previously reported, at interim analysis. Overall, passive surveillance results were similar between groups. The most commonly reported NOADs were inflammatory bowel diseases (incidence per 100,000 person-years: 47.9 [HPV], 59.8 [HBV]) and coeliac disease (16.0 [HPV], 21.8 [HBV]), type-1 diabetes mellitus (22.4 [HPV], 38.1 [HBV]), and juvenile idiopathic arthritis (14.4 [HPV], 16.3 [HBV]). The respective incidence rates for SAEs possibly related to vaccination, throughout the study, were comparable between groups (39.1 [HPV] and 39.8 [HBV]). There were 1252 pregnancies (728 in HPV group, 524 in

HBV group); of these, 31.9% (HPV) and 30.7% (HBV) resulted in live infant outcomes, 59.2% (HPV) and 59.9% (HBV) ended up in elective termination, 8.0% (HPV) and 7.8% (HBV) in spontaneous abortion, all with no apparent congenital anomalies. Anti-HPV-16 geometric mean titre (GMT) was 2588 (95% CI: 2433, 2753) vs 2735 (95% CI: 2420, 3091) and anti-HPV-18 GMT was 878 (95% CI: 818; 942) vs 838 (95% CI: 731, 962) in girls vs boys, respectively.

Conclusion

The HPV-16/18 AS04-adjuvanted vaccine was well tolerated and outcomes were in line with the known safety profile of this vaccine. Immunogenicity results at age ~18.5 years were similar in girls and boys. Health registries contributed an important number of NOAD and pregnancy cases and play a major role in long-term post-vaccination safety surveillance.

Funding: GlaxoSmithKline Biologicals SA

OC 13-12

SAFETY PROFILE OF THE 9-VALENT HPV VACCINE: A COMBINED ANALYSIS OF SEVEN PHASE III CLINICAL STUDIES

E. Moreira¹, **E. Joura**², **P. Van Damme**³, **A. Schilling**⁴, **P. Kosalaraksa**⁵,
E. Moeller⁶, **M. Ritter**⁶, **J. Martin**⁶, **A. Luxembourg**⁶

¹Oswaldo Cruz Foundation, ²University of Vienna, ³University of Antwerp,
⁴Universidad del Desarrollo, ⁵Khon Kaen University, ⁶Merck Sharp & Dohme,
Corp.

Background / Objectives

The 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine includes the 4 HPV types (6/11/16/18) in the quadrivalent HPV (qHPV) vaccine and 5 additional oncogenic types (31/33/45/52/58). Here we present the results of an integrated safety analysis from data gathered over seven Phase III clinical trials of the 9vHPV vaccine.

Methods

Overall, 90.6% of subjects who received 9vHPV vaccine reported an AE. Most adverse experiences were injection-site AEs (84.8%) and the most common were pain (83.2%), swelling (36.1%), and erythema (30.8%). The most common vaccine-related systemic AEs were headache (13.2%), pyrexia (6.1%), nausea (3.2%), dizziness (2.3%), and fatigue (1.9%). Seven SAEs (<0.1%) were determined to be related to 9vHPV vaccine. Few subjects (0.1%) discontinued due to an AE. There were no deaths related to the 9vHPV vaccine.

Results

Overall, 90.6% of subjects who received 9vHPV vaccine reported an AE. Most adverse experiences were injection-site AEs (84.8%) and the most common were pain (83.2%), swelling (36.1%), and erythema (30.8%). The most common vaccine-related systemic AEs were headache (13.2%), pyrexia (6.1%), nausea (3.2%), dizziness (2.3%), and fatigue (1.9%). Seven SAEs (<0.1%) were determined to be related to 9vHPV vaccine. Few subjects (0.1%) discontinued due to an AE. There were no deaths related to the 9vHPV vaccine.

Conclusion

These analyses demonstrate that the IM administration of 9vHPV vaccine is generally well tolerated. Its AE profile was similar to that of qHPV vaccine; injection-site AEs were more common with the 9vHPV vaccine than with the qHPV vaccine (most injection-site AEs were mild to moderate in intensity). Discontinuations were rare and no safety signals of clinical concern were identified.

OC 13-13

END OF STUDY EFFICACY FOR VULVOVAGINAL DISEASE OF A NOVEL 9-VALENT HPV L1 VIRUS-LIKE PARTICLE VACCINE IN 16-26 YEAR OLD WOMEN

E. Joura¹, S. Garland², A. Giuliano³, O. Bautista⁴, J. Chen⁴, E. Moeller⁴, M. Ritter⁴, A. Luxembourg⁴

¹Medical University of Vienna (Austria), ²University of Melbourne (Australia), ³Moffitt Cancer Center (United States of America), ⁴Merck Sharp & Dohme, Corp. (United States of America)

Background / Objectives

An efficacy and immunogenicity study of an investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine was conducted in women 16-26 years of age to demonstrate immunological non-inferiority of HPV 6/11/16/18 response and efficacy against HPV 31/33/4/52/58-related disease. The report presents efficacy against vulva-vaginal disease through end-of-study (i.e. up to month 54 visit).

Methods

14,204 healthy 16-26 year-old women were enrolled into an international, double-blind efficacy and immunogenicity study of the 9vHPV vaccine. Subjects received 9vHPV vaccine or quadrivalent HPV (qHPV) vaccine as a series of injections at day 1/month 2/month 6. Primary analyses included subjects who were seronegative at day 1 and PCR negative from day 1 through month 7 for the HPV type being analyzed. Gynecological examinations were performed every 6 months, and abnormal areas were biopsied.

Results

12,021 women were eligible for this analysis. Efficacy against vulvovaginal disease caused by HPV 6/11/16/18 was equal to qHPV vaccine. Efficacy against HPV 31/33/45/52/58-related VIN/VaIN (any grade) in the primary analysis was 94.4% (95% CI: 67.7, 99.7). No case of high-grade vulvovaginal disease related to the 5 new types was observed in the 9vHPV vaccine group and 3 cases were observed in the qHPV vaccine group. The number of external genital biopsies related to HPV 31/33/45/52/58 was reduced by 92.3% (95% CI: 72.4-98.7).

Conclusion

The 9vHPV vaccine was highly efficacious in preventing HPV 31/33/45/52/58-related vulvovaginal disease up to month 54 visit. Efficacy against disease caused by HPV 6/11/16/18 was the same as with the qHPV vaccine.

OC 13-14

ESTIMATING THE COST-EFFECTIVENESS OF A UNIVERSAL VACCINATION PROGRAMME WITH A NONVALENT HPV VACCINE IN ITALY

F.S. Mennini¹, G. Baio², D. Anger³, C. De Waure⁴, P. Bonanni⁵

¹Faculty of Economics, Centre for Economic and International Studies (CEIS)-Economic Evaluation and HTA (EEHTA), University of Rome (Italy), ²University College London (United kingdom), ³MAPI (France), ⁴Catholic University of Rome (Italy), ⁵Universita degli Studi di Firenze (Italy)

Background / Objectives

A new human papillomavirus (HPV) vaccine protects against the 9 HPV types responsible for 90% of cervical cancers and 90% of HPV related anal cancers. It includes the 4 types contained in the quadrivalent (HPV 6,11,16,18) and 5 additional high-risk oncogenic HPV types (HPV 31, 33, 45, 52, 58). This analysis aims to estimate the public health impact and the incremental cost-effectiveness of a universal (girls and boys) vaccination program with a nonavalent HPV vaccine as compared to a girls only (S1) or universal (S2) vaccination program with a quadrivalent HPV vaccine in Italy.

Methods

A dynamic transmission model including health and cost outcomes related to cervical, anal, vulvar, vaginal diseases and genital warts was calibrated to Italian epidemiological data. The clinical impact due to the 5 new types was included for cervical and anal diseases only. In the base case, a two-dose schedule, lifelong vaccine protection and a vaccination coverage rate of 71% for the 12-year old cohorts were assumed. Ex-factory price of €104 for the quadrivalent vaccine and a theoretical price of €120 for the nonavalent vaccine (corresponding to the public price in the US) were assumed. A threshold of 30,000€/QALY-gained was considered. Deterministic sensitivity analyses on key parameters (such as vaccine price, duration of protection, discount rate) were conducted. No cross protection was considered for the strategies using the quadrivalent vaccine.

Results

Over 100 years, the implementation of a universal vaccination programme with a nonavalent vaccine could avert 22.640 cervical cancers, 275.717 CINs, 8.111 anal cancers and 1.866.845 genital warts compared to S1. This would correspond to

decrease with S1 the vaccine-type related incidence of cervical cancer and precancerous lesions by 81% vs 63%, of anal cancer, by 82% in females vs 68% and 77% vs 42% in males, of genital warts by 55% vs 48% in females and 44% vs 21% with S1 in males and 66%, 75%, 71%, 55% and 44% respectively with S2. The ICER of the switch to a nonavalent universal vaccination was estimated to be equal to 7.165€/QALY when compared to S1 and 10.478€/QALY when compared to S2

Conclusion

The switch to a universal vaccination programme with a nonavalent vaccine in Italy is estimated to be highly cost-effective across a range of sensitivity analyses and is expected to further reduce the public health burden of HPV-related cancers and diseases compared to the current vaccination program.

OC 13-15

Human papillomavirus (HPV) vaccine coverage achievements in thirty low and middle-income countries between 2007-2015

K.E. Gallagher¹, N. Howard², S. Kabakama³, U.K. Griffiths², S. Mounier-Jack², M. Feletto⁴, H.E.D. Burchett⁵, D.S. Lamontagne⁶, D. Watson-Jones⁷

¹London School of Hygiene and Tropical Medicine, Clinical Research Department, Keppel St, London, WC1E 7HT, United Kingdom & Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania. (United Kingdom), ²London School of Hygiene and Tropical Medicine, Department of Global Health and Development, 15-17 Tavistock Place, London, WC1H 9SH, United Kingdom (United Kingdom), ³Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania (Tanzania, United Republic of), ⁴PATH, Vaccine Access and Delivery, PO Box 900922, Seattle, WA, 98109, United States of America (United States of America), ⁵London School of Hygiene and Tropical Medicine, Department of Global Health and Development, 15-17 Tavistock Place, London, WC1H 9SH, United Kingdom. (United Kingdom), ⁶PATH, Vaccine Access and Delivery, PO Box 900922, Seattle, WA, 98109, United States of America (United Kingdom), ⁷London School of Hygiene and Tropical Medicine, Clinical Research Department, Keppel St, London, WC1E 7HT, United Kingdom & Mwanza Intervention Trials Unit, National Institute for Medical Research, PO Box 11936, Mwanza, Tanzania. (Tanzania, United Republic of)

Background / Objectives

Since 2007, HPV vaccine has been available to LMICs for small-scale pilot 'demonstration projects', or national programmes, through manufacturer donations, the GARDASIL® Access Program, Gavi The Vaccine Alliance, or other means. We analysed coverage achieved in HPV vaccine demonstration projects and national programmes that had completed at least one year of implementation between January 2007-January 2015.

Methods

A mapping exercise identified 37 LMICs with HPV vaccine delivery experience. Estimates of coverage and factors related to high coverage were obtained from a systematic published literature search of 5 databases that identified 41 relevant full texts and 9 conference abstracts, 124 solicited unpublished documents, including coverage surveys, and 27 key informant interviews. Coverage achievements were analysed descriptively against country or project/programme characteristics.

Results

Estimates of dose 1 uptake, completion rates and/or final dose coverage were available from 30 of 37 LMICs (45 demonstration projects and 4 national programmes). Only 10 estimates from 10 countries were from surveys; most were administrative estimates. All final dose coverage estimates were over 50%; the majority between 70-90%. There was no correlation between year of delivery of dose 1 and coverage. Dose 1 uptake was generally high across different delivery strategies; however, strategies that included schools attained higher average final dose coverage than health facility-based strategies. In countries with school enrolment rates below 90%, inclusion of strategies to reach out-of-school girls contributed to higher coverage compared to school-only strategies. Among school-based strategies, higher coverage was observed when the Ministry of Education was involved in both planning and implementation of vaccination. Other important factors were political commitment, intensive social mobilisation, community engagement and timely delivery of vaccine within one school year.

Conclusion

While the heterogeneity in data quality, funder requirements, project/programme organisation and design precluded multivariate analysis; this is the most comprehensive descriptive analysis of HPV vaccine coverage in LMICs to date. It is possible to deliver HPV vaccine with excellent coverage in LMICs. Planning and delivery strategies can affect coverage. School-based delivery strategies provide high coverage. Ministry of Education participation, and strategies to vaccinate out-of-school girls are also essential. Social mobilisation should be intensive and all doses should be delivered within one school year.

OC 13-16

HPV Vaccination Coverage at 2 years of Initiating the National Vaccination Programme for Chilean Girls

A. Schilling¹, C. Gonzalez², F. Muñoz²

¹Facultad de Medicina Clínica Alemana-Universidad del Desarrollo (Chile),

²Programa Nacional de Inmunizaciones - Ministerio de Salud (Chile)

Background / Objectives

Chile has the lowest age-standardized incidence and mortality rates of cervical cancer in South America: 12,8 and 6 per 100.000 women per year respectively; largely due to a long time ongoing Cervical Cancer - Early Detection Program and free cervical cancer treatment.

Cervical cancer is the sixth cause of cancer death for Chilean women after breast, gallbladder, lung, stomach and colorectal/anus cancer with an annual crude mortality rate of 8,3 per 100.000 women. It constitutes however the second cause of cancer deaths in women aged 15 – 44 years with an annual crude mortality rate of 2,6 per 100.000 women, only behind breast cancer.

Nevertheless, the screening program (Cytology every 3 years for women 25 - 64 years old) that peaked a 65% coverage between the years 2000 - 2005 has been decreasing since then with an actual percentage of 59-60%, with great differences between geographic regions and/or socioeconomic groups.

Considering this facts, plus the knowledge that HPV 16/18 account for an 85% of all cervical cancers in Chile, the National Ministry of Health decided to introduce HPV vaccination Program for all Chilean girls. The program is free of charge, school-based and was started in 2014.

Methods

The quadrivalent HPV vaccine Gardasil in a 2-dose schedule at month 1 & 12 was chosen because it better addressed Chile's necessities; first dose being given to girls in 4th grade (9 – 10 years old) and second dose at 5th grade (10 – 11 years old).

During years the 2015 and 2016 a catch-up program for girls in 6th and 7th grade is also being conducted.

Results

During the year 2014, a total of 107.392 girls received their first dose of Gardasil. Of them, the 89.5% received the second dose during the year 2015.

During the year 2015, a total of 386.384 Chilean girls got vaccinated both in the routine and the catch-up vaccination:

Vaccination year 2015				
type of vaccination	Routine	Routine	Catch-up	Catch-up
Grade	4th	5th	6th	7th
Dose	1	2	1	1
total girls (n)	112.820	114.160	116.877	120.566
vaccinated girls (n)	95.987	96.116	96.398	97.883
vaccinated girls (%)	85,1	84,2	82,5	81,2

Conclusion

The coverage rate of the HPV vaccination has been as expected, due to the long ongoing tradition for school-based vaccination in the country.

Nevertheless, also in our country anti-vaccine groups are growing stronger, so an effort has to be made to reassure the Chilean families about the benefits and safety of vaccinating their girls against HPV.

References

Bruni L, Barrionuevo-Rosas L, Albero G, Aldea M, Serrano B, Valencia S, Brotons M, Mena M, Cosano R, Muñoz J, Bosch FX, de Sanjosé S, Castellsagué X. ICO Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Chile. Summary Report 2015-12- 23. [Data Accessed 8-3-2016]

Chilean Ministry of Health

Chilean Ministry of Education

OC 13-17

COST-EFFECTIVENESS EVALUATION OF THE QUADRIVALENT HPV VACCINATION PROGRAM FOR FEMALES AGE 11-12 YEARS IN THAILAND

W. Termrungruenglert¹, N. Khemapech¹, N. Havanond¹, P. Deebukkham², A.S. Kulkarni³

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Thailand (Thailand), ²MSD Thailand (Thailand),

³Center for Observational and Real World Evidence, Merck, USA. (United States of America)

Background / Objectives

To examine cost effectiveness of quadrivalent human papillomavirus (HPV) vaccine compared with a cervical cancer screening only program in Thailand.

Methods

Previously published dynamic transmission model was adapted and calibrated for Thailand. The natural history of cervical cancer and genital warts in Thailand was simulated by the HPV model. The model was assessed under the assumption that the HPV vaccination program would be combined with current cervical cancer screening in Thailand. The model assumed that 95% of girls 11-12 years would receive two doses of HPV vaccine. The relative effectiveness of two doses of the quadrivalent HPV vaccine was assumed to be the same as three doses. Only the impact of HPV types 6/11/16/18 was considered for this model. The quadrivalent HPV vaccination program (combined with cervical cancer screening) was compared with a cervical cancer screening only program. Life-long duration of protection was assumed for the quadrivalent HPV vaccine.

Results

The quadrivalent HPV vaccines resulted in the reduction of HPV types 6/11 related genital warts in females (85%) and males (82%), CIN1 (82%); HPV 16/18 related cervical cancer (61%), CIN2/3 (74%), and CIN1 (74%) over a 100 year time horizon. Considering the recommended threshold of 1.2 GNI or 185,898 THB/QALY (4,712 Euro/QALY) for Thailand, the implementation of the quadrivalent vaccination program was cost effective as compared to the cervical cancer screening only program with discounted incremental cost-effectiveness ratios (ICER) of 26,901 THB/QALY (682 EURO/QALY).

Conclusion

In Thailand, vaccinating 11-12 year old girls with the quadrivalent HPV vaccine has additional public health impact and is cost effective as compared with the cervical cancer screening only program. The results support decision-making process to include HPV vaccine to the national vaccination program.

OC 14-01

RISK OF CERVICAL CANCER AFTER A NEGATIVE SMEAR BY AGE

I.M.C.M. De Kok¹, K. Rozemeijer¹, S.K. Naber¹, S.M. Matthijsse¹, E.E.L. Jansen¹, H.M.E. Van Agt¹, L.I.H. Overbeek², A.G. Siebers², F.J. Van Kemenade³, M. Van Ballegooijen¹

¹Erasmus MC, University Medical Center, Department of Public Health, Rotterdam, The Netherlands (Netherlands), ²PALGA, the Nationwide network and registry of histo- and cytopathology in the Netherlands, Houten, the Netherlands (Netherlands), ³Erasmus MC, University Medical Center, Department of Pathology, Rotterdam, The Netherlands (Netherlands)

Background / Objectives

There are indications that the test sensitivity of the Pap smear changes with age. The incidence of clinical cervical cancer after a negative test result reflects the sensitivity of the test. In this study, we examined the risk of cervical cancer after a negative Pap smear by different age groups.

Methods

Screening history data (from the Dutch nationwide registry of histo- and cytopathology (PALGA)) have been linked on an individual level to cervical cancer incidence data from the Netherlands Cancer Registry (NKR). All primary negative screening smears taken from 1996 to 2007 were analyzed, stratified by seven age groups (29-33, 34-38, ..., 59-63 years). The follow-up period after a negative smear was 72 months. Cox regression analyses were performed to assess the hazard ratio (HR) for cervical cancer after a negative smear, adjusted for calendar time and screening history.

Results

792 cervical cancers were diagnosed in 17,247,925 women-years after a negative test result. We found that older women have a significantly decreased risk to be diagnosed with cervical cancer after a negative Pap smear compared to younger women. With increasing age, the risk of cervical cancer after a negative smear decreased. Compared to women of 29-33 years, the HR was 0.84 (95% CI: 0.66-1.07) for 34-38 years, 0.65 (95% CI: 0.50-0.84) for 39-43 years, 0.43 (95% CI: 0.32-0.58) for 44-48 years, 0.37 (95% CI: 0.27-0.51) for 49-53 years, 0.42 (95% CI: 0.31-0.58) for 54-58 years, and 0.33 (95% CI: 0.23-0.48) for 59-63 years.

Conclusion

Older women have a significantly lower risk to be diagnosed with cervical cancer after a negative Pap smear compared to younger women. The lower risk might be caused by a higher test sensitivity in older women to detect relevant cervical lesions by cytological screening, or by less rapid development of cancer in older women. Our results indicate that a longer interval at older ages might be more (cost-)efficient to prevent cervical cancer in the population.

OC 14-02

CERVICAL CANCER MORTALITY IN UN(DER)SCREENED WOMEN IN THE NETHERLANDS

S. Naber¹, **K. Rozemeijer**¹, **S. Matthijsse**¹, **E. Jansen**¹, **H. Van Agt**¹, **L. Overbeek**², **A. Siebers**², **F. Van Kemenade**³, **M. Van Ballegooijen**⁴, **I. De Kok**⁴

¹1) Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (Netherlands), ²2) PALGA, the Nationwide network and registry of histo- and cytopathology in the Netherlands, Houten, the Netherlands (Netherlands), ³3) Department of Pathology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (Netherlands), ⁴4) Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands (Netherlands)

Background / Objectives

In the Netherlands, five-year screening coverage is approximately 75%. A significant proportion of cervical cancer cases are diagnosed in women who recently refused or delayed screening participation. By examining the screening history of women who died from cervical cancer, we investigated whether the same is true for cervical cancer mortality.

Methods

Individual screening histories from the Dutch nationwide registry of histo- and cytopathology (PALGA) were linked to mortality data from the Netherlands Cancer Registry. All cervical cancer deaths between January 1996 and October 2009 were selected. Based on birth year and age, we derived whether women were recently (i.e. ≤ 7 years), not recently (i.e. > 7 years) or never invited for screening. Screening histories were used to examine whether women were actually screened.

Results

From the 2,889 women who died from cervical cancer, 814 (28.2%) were considered never invited for screening as they were born before 1925 and were over 53 years of age in 1978, when a nationwide screening program was introduced in the Netherlands. Another 81 (2.8%) deaths from cervical cancer could not have been prevented by screening because these women were diagnosed either before or during the year in which they were first invited for screening (2.0% and 0.8%,

respectively). Another 396 women were not recently invited for screening because of their age (>67 years). From the 1,598 (55.3%) women who were diagnosed in the screening age range (31-67 years), 606 (21.0%) were recently screened, 226 (7.8%) were not recently screened, and 766 (26.5%) were never-screened.

Conclusion

From the women who were diagnosed with cervical cancer within the screening age range, 62% were not screened within the last 7 years. As in general only 25% of women are not recently screened, cervical cancer mortality risk was approximately 5 times $((0.62/0.25)/(0.38/0.75))$ higher in women who did not (fully) comply with screening guidelines than in those who did. Increased awareness of this elevated mortality risk among non-participating women might increase their willingness to participate in screening.

OC 14-03

EFFECT OF ORGANIZED SCREENING AND OPPORTUNISTIC TESTING IN CERVICAL CANCER IN FINLAND AMONG YOUNG WOMEN

P. Makkonen¹, S. Heinävaara², T. Sarkeala³, A. Virtanen³, A. Anttila³

¹The Finnish Cancer Registry, Faculty of Medicine, University of Helsinki (Finland), ²The Finnish Cancer Registry, The Department of Public health, University of Helsinki (Finland), ³The Finnish Cancer Registry (Finland)

Background / Objectives

Effectiveness of organized cervical cancer screening has been shown in several studies. However, screening under 25 years old women has been shown to have little or no impact on the risk of cervical cancer and clear effects have been observed above 40 years. In Finland an extensive opportunistic screening practice that concentrates especially on younger women exists alongside the national screening programme. However, the significance of the opportunistic testing in preventing cervical cancer is unclear. The aim of this study is to clarify the effect of opportunistic testing and organized screening on the risk of cervical cancer among young women in Finland.

Methods

There were 462 cervical cancer cases screened and diagnosed among women aged below 40 years and in 2000-2009 in the Finnish Cancer Registry. Screening histories for these women and their 2,772 age-matched controls were derived by linkage to the mass screening register. The data was further linked with opportunistic testing data available for 29% of the cases and 34% of the controls. The opportunistic data includes Pap smears taken in the public primary health care covering the southern parts of Finland (Turku and Uusimaa region), Pap smears taken in the student health care and Pap smears reimbursed in the private sector covering the whole country. OR's and 95% confidence intervals for the association of cervical cancer diagnosis and participation in organized screening and opportunistic testing 0,5-5,5 years before the diagnosis were estimated using unconditional logistic regression. The results were not yet corrected for self-selection bias.

Results

OR of cervical cancer for screening below age 25 was 0.93 (95% CI 0.31-2.81). All smears in that age group were from opportunistic testing. Participation in organized

screening at 25 to 40 resulted in OR 0.52 (0.36-0.75), participation only in opportunistic testing 0.75 (0.49-1.14) and participation in both organized screening and opportunistic testing 0.44 (0.24-0.81).

Conclusion

According to initial results opportunistic testing showed no clear additional benefit on preventing cervical cancer. The study also supports previous findings about the lower effect of screening in younger age groups compared to older ones. Taking into account the high costs of screening and related CIN treatments in young women, current screening practice in Finland should be questioned and revised.

OC 14-04

Impact of Cytology Lab Service Delivery on the Cervical Health Screening Algorithm

K. Valentine¹, D. Vanden Broeck¹, S. Weyers², M. Arbyn³, J.P. Bogers⁴

¹International Centre for Reproductive Health, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium (Belgium), ²Department of Gynaecology and Obstetrics, Ghent University Hospital, Ghent, Belgium (Belgium), ³Unit of Cancer epidemiology, Scientific Institute of Public Health, Brussels, Belgium (Belgium), ⁴National Reference Center for HPV, Brussels (Belgium)

Background / Objectives

To assess and analyze the quality aspects between cytology labs, focusing on quality of samples, satisfactory/unsatisfactory results, TAT from testing sample to result and the variance between reporting per cytcategory.

Methods

SEHIB (Surveillance of Effects of Human Papillomavirus Immunization in Belgium) was a study to mainly generate baseline data at introduction of the prophylactic HPV vaccination and to provide a surveillance framework for measuring the impact of HPV vaccination in Belgium. To do this, 8 cytopathology lab were used – 4 universities and 4 peripheral labs. The study started in November 2010 recruiting women (< 30 > years of age) and the last patient was seen July 2014. In total 6,630 specimens were included in the study, 90% of these collected in phase 1 are representative of screening samples and 10% collected in phase 2 were linked to abnormal screening or follow-up samples. Phase 2 samples were only collected in the university laboratories.

Results

Alongside the molecular and cytological analysis performed, key performance indicators of these 8 labs were measured and upon evaluation confirmed substantial localized variances in service delivery and testing among the following topics:

- TAT interval between sample collection and cytological interpretation ranged from a mean of 2.4-13 days.
- A variance in unsatisfactory samples ranged between 0.17-12.36% overall and increased when only evaluating screening Pap smears, 0.26-17.41%.

- Distribution of cervical epithelial abnormalities, comparing NILM to epithelial abnormalities, ranged from 0.62-11.93%.

Conclusion

There are opportunities for service delivery improvements in the following above-mentioned areas. The rates of cervical epithelial abnormalities observed, although in line with international benchmarks, show substantial localized discrepancies. This alongside the subjectivity and variance in diagnosis of NILM versus epithelial abnormalities questions the robustness and the quality assessment of cytological cervical screening strategy.

OC 14-05

CUMULATIVE PROBABILITY OF ABNORMALITIES IN ORGANIZED CERVICAL CANCER SCREENING

M. Pankakoski, S. Heinävaara, T. Sarkeala, A. Anttila

Mass Screening Registry/Finnish Cancer Registry, Unioninkatu 22, FI-00130, Helsinki (Finland)

Background / Objectives

In Finland women are invited to cervical cancer screening every five years between the ages 30—60, with some municipalities also inviting 25 and 65-year-olds. Thus a woman may go through up to 9 routine screens during her lifetime. If borderline abnormalities are detected, additional follow-up screening is recommended. The purpose of this study was to determine the cumulative probability of having any cytological abnormalities by the age of 64 in organized cervical cancer screening. The focus of attention was particularly on the difference between borderline and more severe abnormalities. We also analyzed whether previous abnormalities were associated with the risk of detecting new ones.

Methods

Individual screening histories during 1991—2012 were collected for all women from the Mass Screening Registry of the Finnish Cancer Registry. Analysis of cumulative probabilities was restricted to cohorts born in 1950-1965 since they had the most comprehensive follow-up data with 1 207 017 routine screens and 88 143 follow-up screens among 364 487 women. The most severe result within a five-year screening round was treated as the outcome, detected either by routine or follow-up screening. Probabilities by age were estimated using logistic regression with a GEE approach which accounts for individual-level correlation.

Results

The probability of experiencing any abnormality at least once by the age of 64 was 34.0% (95% CI: 33.3-34.6%). This was considerably high compared to the proportion of results warranting referral (5.4%, 95% CI: 5.0-5.8%) or results with histologically confirmed findings (2.2%, 95% CI: 2.0-2.4%). Previous occurrences were associated with an increased risk of detecting new ones, specifically in older cohorts.

Conclusion

The difference in the magnitude between mild and more severe results detected by the programme refers to a notable overdiagnosis of borderline results which may increase costs and have psychological downstream consequences. Improvement of diagnostic criteria concerning borderline abnormalities should reduce the problem. The overall detection of pre-cancerous lesions was very low, indicating that a proportion of pre-cancerous lesions might be treated outside the programme.

OC 14-06

AUDIT OF SCREENING HISTORIES AND EFFECTIVENESS OF SCREENING

S. Lönnberg¹, I. Baasland², P.R. Romundstad²

¹Cancer Registry of Norway, Oslo (Norway), ²Norwegian University of Science and Technology, Trondheim (Norway)

Background / Objectives

Screening programme audits are recommended in the European Guidelines and considered an ethically required part of population-based screening. We conducted a systematic programmatic audit in order to identify the magnitude of different screening failures and their impact on the remaining cervical cancer burden in Norway.

Methods

The case population consisted of all invasive cancers recorded in the Cancer Registry of Norway with incidence dates in 1993-2013, and amounted to 6467 cases. Ten age-matched controls per case were drawn from the National Population Register. Cases and controls were individually linked to the screening databases for screening history analysis and categorisation. There were 17 case and 121 control women who had opted-out of screening registration and these were excluded from further analysis for a final dataset of 6450 cases and 64,549 controls. Screening exposure variables were constructed using both three and five year windows of exposure. Cervical tests in the last 6 months before diagnosis were considered diagnostic, non-preventive, and therefore discounted. Screening history was categorised by participation, result of primary screening test and management of positive tests. Odds ratios for cervical cancer according to screening history were calculated with 95% confidence intervals (CIs).

Results

Most, 63%, of the case women had not participated in screening in the three-year interval ending 6 months before diagnosis, compared with 47% of the controls. Corresponding proportions for a five-year interval was 53 and 37%. The risk of cervical cancer was similarly reduced among women that had participated in screening in either a three- (OR 0.47, 95% CI 0.45-0.50) or five-year interval (OR 0.45, 95% CI 0.43-0.48). Of the cases, 22% had a negative last primary cytology in the three-year interval, compared with 50% of the controls. The risk of cervical cancer was low among these women compared to non-participants (OR 0.30, 95% CI 0.28-

0.32). Among women screened within the recommended three-year interval, the risk for cervical cancer was much higher for those with a borderline (OR 11.5, 95% CI 10.3-12.8) or high-grade primary cytology (OR 18.0, 95% CI 15.2-21.3) compared with those with normal smears. Women with borderline and high-grade primary cytology constituted 10 and 4.3% of all women with cervical cancer, compared with only 2.0 and 0.5% of the controls.

Conclusion

Non-participation was the most important contributor to cervical cancer risk in Norway. However, women with positive screening tests had significantly elevated risks of cervical cancer, indicating possibilities for improvement also in the management and treatment of screen-positive women.

OC 14-07

NINE YEARS EXPERIENCE IN 412.000 CASES: LIQUID BASED CYTOLOGY AND COMPUTERASSISTANCE COMPARED TO CONVENTIONAL CYTOLOGY

H. Ikenberg, B. Pittel, M. Faber, A. Bernhardt, I. Singh, B. Christoph, X. Arjola

Cytomol, Laboratory for Cytology and Molecular Biology, D-60437 Frankfurt (Germany)

Background / Objectives

Several major studies showed inconsistent results for the comparison of liquid-based cytology (LBC) with conventional cytology (CC). However, some trials found a significantly higher sensitivity for HSIL (high grade intraepithelial lesions) with the computer-assisted ThinPrep-Imaging-System (TIS) compared to conventional cytology (CC) and even manually read ThinPrep LBC. Here we report the performance of TIS compared with CC in women who participate in the German cervical cancer screening program.

Methods

At Cytomol, a commercial lab specialized in cervical cancer prevention, since 2000 an experience with over 450.000 LBC cases has been achieved. Since 2007 all LBC specimens have been processed by TIS. In Germany LBC is reserved to privately insured and self-paying patients while public healthcare only reimburses CC. To avoid bias we limited this analysis to privately insured patients. Finding rates of cytologic abnormalities with TIS and CC were compared. Cytologic diagnoses originally reported in the Munich Nomenclature II (MN; with the use of the inofficial Pap IIW category) until 30.6.2014, from then in the MN III (which is still the reporting standard in Germany) were translated to TBS (The Bethesda System).

Results

From 2007 to 2015 412.585 slides have been analyzed among them 286.161 by TIS and 126.424 with CC. Except of extremely bloody and very cell-rich probes 97.25% of the smears were accepted for analysis by TIS. TIS had a rate of LSIL (low grade intraepithelial lesions; MN III: Pap IIID1) of 2.04% compared to 0.51% for CC, an increase of 300%. HSIL (MN III: Pap IIID2 + Pap IVa/b) was found in 1.14% with TIS vs 0.34% with CC (+225%). The ASC-US rate (MN III: Pap II-p + III) was 2.60% with TIS and 1,31% with CC, an increase of 101% which is much lower than the rise in

LSIL and HSIL. It is therefore suggestive that the higher sensitivity of TIS was achieved without lowering specificity. All these results remained stable over the 9 years analyzed. With TIS 20.4 slides/h were screened, compared to 12.2 for manually read TPs and 8.0 with CC. However, the technical expenditure for TIS was much higher.

Conclusion

In long-time routine use of a commercial lab computer-assisted LBC with the ThinPrep-Imaging-System provided higher sensitivity and higher productivity without lower specificity at the cost of higher technical expenditure.

OC 14-08

Liquid-based cytology and human papillomavirus testing in the cervical screening programme in Luxembourg

A. Latsuzbaia¹, G. Hebette¹, M. Fischer¹, M. Arbyn², S. Weyers³, P. Vielh¹, F. Schmitt¹, J. Mossong¹

¹Laboratoire National de Santé (Luxembourg), ²Scientific Institute of Public Health (Belgium), ³Ghent University Hospital (Belgium)

Background / Objectives

Luxembourg does not have an organised cervical screening programme and screening has historically been conducted in a single national cytology laboratory. In 2014, the liquid-based ThinPrep® Pap Test and Imaging System (TPTIS) by Hologic replaced conventional cytology for cervical screening at this national facility. At the same time, human papillomavirus (HPV testing) using the Aptima HPV test was introduced, mainly on samples showing atypical squamous cells of undetermined significance (ASC-US) or upon physician's request. The aim of our study was to estimate the prevalence of abnormal cervical cytology, of high risk HPV (hrHPV) infection and their correlation among screened women in Luxembourg.

Methods

From July 2014 until December 2015, 163,321 cervical samples from 121,027 women (mean age 42.2 years) were investigated by the national cytology laboratory in Luxembourg. Slides were prepared according to manufacturer's instruction using the TPTIS methodology (Hologic Inc., Bedford, MA). Only the first sample of each woman assessed during the study period was considered for computation of the prevalence of cytological abnormalities as well as the prevalence of HPV per cytological category and by age.

Results

The prevalence of abnormal cervical cytology was as follows: ASC-US 1.6 %, low-grade squamous intraepithelial lesion (LSIL) 2.0%, and high-grade squamous intraepithelial lesion (HSIL) 0.4%. Prevalence of LSIL was highest in 20-24 year olds (4.3%), whereas the prevalence of ASC-US and HSIL was highest in 25 -29 year olds (2.6% and 0.8%, respectively). Based on 11,582 samples with concomitant cytology and HPV testing, hrHPV was detected in 9.6%, 45.3%, 70.4%, and 91.9% of women negative for intraepithelial lesions and malignancies (NILM), ASC-US, LSIL and HSIL, respectively. The prevalence of hrHPV was highest in 20-24 year olds (43.5%).

Among 235 HSIL samples, 48.5%, 4.3%, and 39.2% were positive for HPV 16, HPV 18/45 or other hrHPV, respectively; among NILM samples, the prevalences for HPV 16, HPV 18/45 and other hrHPV were 1.4%, 0.5% and 7.7%, respectively.

Conclusion

Our study showed a strong correlation between cytology and hrHPV. The prevalence of hrHPV infections in patients with HSIL (91.9%) and LSIL (70.4%) was similar to that observed in Belgium [1]. Our study provides important information to evaluate the prevention of cervical cancer in Luxembourg, particular in view of switching to primary HPV testing and for monitoring the future impact of HPV vaccination.

References

1. Arbyn M, Haelens A, Desomer A, Verdoodt F, Thiry N, Francart J, Hanquet G, Robays J. Cervical cancer screening program and human papillomavirus (HPV) testing, part ii: update on HPV primary screening – Summary. Health Technology Assessment (HTA) Brussels: Belgian Health Care Knowledge Centre (KCE). 2015. KCE Reports 238Cs. D/2015/10.273/16.

OC 14-09

EVALUATION OF ORGANISED PRIMARY HPV SCREENING OF WOMEN AGED 30 - 64 IN SWEDEN

C. Eklund¹, **H. Lamin**², **P. Olausson**³, **A. Carlsten-Thor**⁴, **M. Elfström**¹, **S. Törnberg**⁴, **J. Dillner**¹

¹Dept of Laboratory medicine, Karolinska Institutet (Sweden), ²Dept of Laboratory medicine, Karolinska University Hospital (Sweden), ³Dept of Medical Epidemiology and Biostatistics, Karolinska Institutet (Sweden), ⁴Regional Cancer Center, Cancer Screening Unit, Stockholm (Sweden)

Background / Objectives

We evaluated the effectiveness of primary HPV screening for women aged 30-64 when introduced into an organised, population-based cervical screening program in Sweden.

Methods

HPV based primary screening was implemented in the organized screening program in the greater Stockholm county in June 2014, randomising 50% of all resident women 30-60 years to either primary HPV screening with cytology triage or to primary cytology, in 2015 the age was extended to 30-64 years. HPV+/Cyt- women in the screening ages are referred to the next round of organised screening, whereas HPV+/Cyt- women aged 64 (who otherwise would have been acquitted from the programme) will continue to be screened. The primary evaluation is the sensitivity for CIN2+ detection and cost-effectiveness of the new policy in relation to the previously used policy

Results

68674 primary HPV test were performed starting in June 2014 to December 2015. The population HPV prevalence was 8,3 % and there was a 91,7 % decline in number of cytologies performed. During 2014, 26539 women attended any of the two arms of the screening program. 0,25 and 0,27 % of the women were diagnosed with CIN2+. No women with persisting HPV have yet been referred to colposcopy therefore a similar number of CIN2+ in both arms are the expected results.

Conclusion

Primary HPV screening is acceptable to the population, results in higher attendance rates and reduces the screening costs.

OC 14-10

PARALLEL TESTING FOR HIGH-RISK HPV AND LIQUID BASED CYTOLOGY IN PRIMARY SCREENING FOR CERVICAL CANCER

J.E. Levi¹, T.R. Martins¹, A. Longatto-Filho², D.D. Cohen³, L.M.S. Fuza³, L. Cury³, L.L. Villa⁴, J. Eluf-Neto⁵

¹Instituto de Medicina Tropical da Universidade de São Paulo (Brazil), ²USP's College of Medicine (Brazil), ³Fundação Oncocentro de São Paulo (Brazil), ⁴National Institute of Science and Technology of the Diseases Associated to the Papillomavirus (Brazil), ⁵Fundação Oncocentro de São Paulociated to the Papillomavirus (Brazil)

Background / Objectives

Every year there are approximately 16,000 new cases of cervical cancer in Brazil. New screening technologies may lead to a reduction of this number by expanding the population coverage but also by improving the detection rate of precursor lesions.

Methods

Women participating in a routine CC primary screening program were invited to enroll in this study. An LBC sample is collected in SurePath medium and transported to Fundação Oncocentro where BD Totalys™ System prepares, in parallel, slides for cytology and an aliquot for the BD Onclarity™ HPV Assay. A positive high-risk HPV test and/or cytology class > ASC-US refers the patient to colposcopic examination and biopsy, if found necessary by the clinican.

Results

In between Dec., 2014 and Jan. 2016 12,084 women joined this study. Hr-HPV DNA prevalence was 15.2% while cytological abnormalities were verified in 8.9%. Per protocol, 2,058 were referred, but so far only 859 were evaluated by colposcopy. Two-hundred and sixteen were biopsied and 47 CIN2+ cases diagnosed, 46 Hr-HPV DNA+ (98%) and 11 cytology negative (76%). HPV testing identified two squamous carcinomas and an adenocarcinoma which was missed by cytology. 88% of the HSILs were positive for HPV. Hr-HPV DNA frequency among women ≥ 30 yo was 10% and the overall HPV16/18 prevalence was 4.2%. Among the 11 CIN3+ cases HPV 16 was found in 4 and HPV 52 in 3, while HPV 18, 31, 45 and HPV33_58 were identified in one CIN3+

Conclusion

Hr-HPV DNA detected a significant number of patients with premalignant lesions missed by cytology. In another fraction, cytology provided a classification that would, according to the current Brazilian algorithm, delay the CIN2+ detection due to a loop of repeating cytology in 6 mo - 1 year. Screening in Brazil is still mostly opportunistic demanding a “one-stop” final diagnosis. If HPV-DNA screening is adopted, to avoid an increase in the need for colposcopic examinations, it will be very important to add to HPV-DNA+ samples , one marker of high positive predictive value, before referral , preferably from the same primary cervical smear. In addition, the European age cut-off of 30 yo for HPV based-screening is probably not ideal in Brazil, where is not uncommon to observe young women with CIN2+.

OC 14-11

Implementation of HPV-test in primary screening has not decreased the attendance rate in the Norwegian cervical cancer screening programme

B. Engesæter, T. Andreassen, S. Lönnberg, A. Tropé, M. Nygård

Cancer Registry of Norway (Norway)

Background / Objectives

Human Papilloma Virus (HPV) testing is currently implemented in a randomized controlled fashion as the primary test in the Norwegian cervical screening programme. Women are allocated randomly to either HPV test or liquid-based cytology based on their date of birth. The implementation involves women in the age group 34-69 years in four Norwegian counties, counting approximately 285 000 women. The impact of the new screening strategy on the attendance rate of the women receiving an invitation letter was evaluated.

Methods

Systematic information and appropriate organization may be important for the acceptance of new screening strategies, both for health professionals and target women. The Cancer Registry of Norway was responsible for the coordination of the preparations prior to implementation, and two expert groups were established to support the effort. The first group focused on: 1) information to involved parties (the women, the GPs, the gynecologists and the laboratory staff), 2) establishing laboratory infrastructure and procedures, and 3) changes in data flow and invitation/reminder routines at the Norwegian Cancer Registry. The second group concentrated on 1) the scientific safeguard and 2) the development of project evaluation protocols.

Results

One year after project initiation, the logistical implementation of the project is evaluated to be successful. Careful preparation has translated to a smooth transition from cytology to HPV testing with only minor exceptions reported. The IT-solutions for randomization are established in each laboratory, and is under continuous optimization to secure optimal and equal follow-up for every woman. The test results are reported by the laboratories to the Norwegian Cancer Registry, and analysed quarterly. Information has been provided to the women through newspapers, social media, information letters and leaflets. The implementation of a new screening test

has achieved general acceptance, and the screening attendance after invitation letters has been comparable between the women offered HPV test and the women getting a cytological evaluation of their samples, $44.8\% \pm 0.9$ vs $44.0\% \pm 0.8$, respectively.

Conclusion

It is important to prepare a national implementation of HPV based screening in close collaboration with all stakeholders in order to prevent misunderstandings and ensure high quality and optimal security for the individual women at all stages. After thorough preparations and dissemination of information, the implementation of HPV testing did not produce any changes in screening acceptance in Norway.

OC 14-12

RANDOMIZED IMPLEMENTATION OF PRIMARY HIGH RISK HUMAN PAPILLOMA VIRUS TESTING FOR CERVICAL CANCER SCREENING IN NORWAY

M. Nygård¹, B. Engesæter², A. Trope²

¹HPV-related Epidemiological Research Unit, Dept. of Research, Cancer Registry of Norway, Oslo (Norway), ²Cervical Cancer Screening Unit, Cancer Registry of Norway, Oslo (Norway)

Background / Objectives

Improved understanding of the natural history of cervical carcinogenesis, and knowledge of the relative performance of different screening tests implies superiority of the high risk human papillomavirus (hrHPV) test over cytology in cervical cancer screening. Five-year hrHPV testing in primary screening was implemented instead of the standard of care, triennial Pap-smear screening, from February 1st, 2015 in Norway. Updated results from on-going surveillance will be presented in the conference.

Methods

In 2014, the same HPV test, biobank solutions, similar communication strategies etc., were adapted by screening units implementing new cervical cancer screening technology for approximately 1/4 of eligible Norwegian female population (in four counties). Information about the target population, such as date of birth and address is available for administration of the program. Based on date of birth (odd and even days), which equals to randomized allocation, 50% of 34-69 years old women receive cytology and 50% hrHPV test in screening. Every cytology, hrHPV, and histology result is reported to the Cancer Registry of Norway for coordination and surveillance.

Results

By the end of 2015 more than 60,000 women, 34 to 69 years of age, were screened with either hrHPV or cytology in counties implementing hrHPV screening; attendance rates were comparable; the overall hrHPV positivity rate in HPV arm was 6,7%; reflex cytology in triage of hrHPV positives indicated high proportion of abnormalities, leading to about 4% colposcopy referral in HPV screening, twice as in cytology screening; there was significantly more CIN2+ diagnosed in the HPV arm. Altogether, 30 cancers were diagnosed, of which 16 were in the cytology and 14 in the HPV arm.

Conclusion

Careful preparation has translated to smooth transition from cytology to HPV testing. Quality assessment of the cytology smear diagnostics in hrHPV positives might be useful to evaluate the extent of the emerging diagnostic shift, likely to be result because of readily known hrHPV status. Gradual and randomized implementation alleviates workload increase for the coloposcopy and pathology services and allows direct comparison of effect indicators between two screening modalities.

OC 14-13

QUALITY INDICATORS FOR PRIMARY HRHPV SCREENING FOR CERVICAL CANCER

M. Arbyn¹, E. Brouwer², K. Holtzer-Goor², W. Rodenburg³, N. Van Der Veen²

¹Belgian Cancer Centre, Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium (Belgium), ²National Institute for Public Health and the Environment, Centre for Population Screening, Bilthoven, the Netherlands (Netherlands), ³National Institute for Public Health and the Environment, Centre for Health Protection, Bilthoven, the Netherlands (Netherlands)

Background / Objectives

Primary hrHPV screening offers improved protection against cervical cancer compared to cytology-based screening and enables extension of the screening interval. In the Netherlands, this method will be introduced as well as the option for non-responders to receive a self-sampling device. The introduction of the new screening policy requires indicators to be monitored allowing rapid adaptations. Furthermore, indicators for long-term impact are needed. We defined a new set of quality indicators dedicated to primary hrHPV screening for cervical cancer.

Methods

Requirements for quality assurance and impact evaluation of the new population-based screening programme, were defined in a working group "Quality, Monitoring and Evaluation" and were discussed with international experts.

Results

The proposed indicators involve monitoring at different levels including performance of laboratories, accuracy of the chosen screening test and indicators to monitor the entire screen chain (participation of the population using clinician- and self-samples, test positivity and PPV of screen and triage tests, referral rate, compliance with repeat and diagnostic testing). Furthermore, indicators for long-term monitoring on effectiveness were proposed (e.g. are the new test and strategies leading to the planned outcomes). To facilitate evaluation and monitoring databases, new IT configurations were established.

Conclusion

Internationally agreed uniform indicators will enable to adapt timely and improve early steps in the screening and follow-up process of the new screening programme and provide insight in long-term effectiveness of the hrHPV screening in the Netherlands and elsewhere.

OC 14-14

EVALUATION OF COLPOSCOPY AS A DIAGNOSTIC TRIAGE FOR SINGLE VISIT SCREEN AND TREAT STRATEGY IN VIA BASED CERVICAL CANCER SCREENING PROGRAMS IN INDIA.

S. Pimple, G. Mishra, S. Shastri

TATA MEMORIAL CENTRE

Background / Objectives

Poor compliance for screening, diagnosis and treatment due to multiple visits involved is perceived as the single most major barrier for effective cervical cancer programs in resource constrained countries already facing challenges for implementation due to absence of reliable health infrastructure for cytology and HPV testing programs.

Objective: Study was performed to evaluate the efficacy of diagnostic triage by colposcopy compared to conventional cytology and HPV DNA testing in Visual Inspection with 5% Acetic Acid(VIA) based primary screening programs

Methods

257 VIA positive women were offered diagnostic triage with Colposcopy, Conventional cytology and HPV DNA testing. Test characteristics and their 95% confidence intervals were compared with that of conventional cytology and HPV DNA test against the reference standard of histopathology. py compared to conventional cytology and HPV DNA testing in Visual Inspection with 5% Acetic Acid(VIA) based primary screening programs

Results

The sensitivity of diagnostic colposcopy, cytology and HPV DNA by Hybrid Capture II was 0.69(95% CI: 0.41 - 0.89), 0.44(95% CI: 0.20 - 0.70) and 0.69(95% CI: 0.41 - 0.89) respectively and that of specificity was 0.76(95% CI: 0.70 - 0.81), 0.97(95% CI: 0.94 - 0.99) and 0.83(95% CI: 0.78 - 0.88) respectively. Colposcopy and HPV DNA had similar false negative rate (FNR) [0.31(95% CI: 0.11 - 0.59)].

Conclusion

Diagnostic triage for VIA positive women by colposcopy was comparable to HPV DNA testing and was more sensitive than conventional cytology. In settings with limitations in establishing diagnostic cytology and molecular facilities and also difficulty in accessing health-care facilities triage by colposcopy should be considered as a possible alternative.

References

Sankaranarayanan R et al., A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India, *International Journal of Cancer*, 2005;116: 617–623.

Sankaranarayanan R et al., A critical assessment of screening methods for cervical neoplasia, *International Journal of Gynaecology & Obstetrics*, 2005;89:S4–S12.

OC 14-15

BALANCING BENEFITS AND HARMS IN CERVICAL CANCER SCREENING – A DECISION ANALYSIS FOR THE AUSTRIAN HEALTH CARE CONTEXT.

G. Sroczyński¹, E. Esteban², A. Widschwendter³, W. Oberaigner⁴, W. Borena⁵, D. Von Laer⁵, M. Hackl⁶, G. Endel⁷, U. Siebert⁸

¹Department of Public Health, Health Services Research, and Health Technology Assessment, UMIT - University for Health Sciences, Medical Informatics and Technology, Hall i.T./ ONCOTYROL Center for Personalized Cancer Medicine, Innsbruck (Austria), ²Department of Public Health, Health Services Research, and Health Technology Assessment, UMIT - University for Health Sciences, Medical Informatics and Technology, Hall i.T./ ONCOTYROL Center for Personalized Cancer Medicine, Innsbruck (Austria), ³Department of Obstetrics and Gynecology, Medical University Innsbruck, Innsbruck (Austria), ⁴Institute for Clinical Epidemiology, Cancer Registry Tyrol, Tirol Kliniken, Innsbruck (Austria), ⁵Division of Virology, Department of Hygiene, Microbiology, Social Medicine, Medical University of Innsbruck, Innsbruck (Austria), ⁶Statistics Austria, Austrian National Cancer Registry, Vienna (Austria), ⁷Department for Evidence Based Economic Health Care, Main Association of Austrian Social Insurance Institutions, Vienna (Austria), ⁸Department of Public Health, Health Services Research, and Health Technology Assessment, UMIT - University for Health Sciences, Medical Informatics and Technology, Hall i.T./ ONCOTYROL Center for Personalized Cancer Medicine, Innsbruck / Harvard T. H. Chan School of Public Health & ITA, Boston, USA (Austria)

Background / Objectives

Primary cervical cancer screening may be optimized using new risk-based screening and follow-up algorithms with improved benefit-harm trade-offs. Our aim was to systematically evaluate and compare the benefit-harm balance of different cervical cancer primary screening strategies for the Austrian context.

Methods

We used a validated Markov-state-transition model calibrated to the Austrian epidemiological setting and clinical context of the disease to evaluate different screening strategies that differ by primary screening test (including cytology, p16/Ki-67-dual stain, and HPV-testing alone or in combinations), screening interval, age, and specific follow-up algorithms for women with positive test results. Austrian clinical, epidemiological and economic data, as well as test accuracy data from

international meta-analyses and trials were incorporated. Predicted outcomes are reduction in cervical cancer incidence and mortality, remaining life expectancy, overtreatment (defined as conization with histological diagnosis of no lesion or a lesion grade CIN 1), and the incremental harm-benefit ratios (IHBR) measured in numbers of overtreatment per additional prevented cervical cancer death. Comprehensive sensitivity analyses were performed.

Results

Based on our results, within the same screening interval, HPV-based primary screening strategies are more effective compared with cytology or with p16/Ki-67-testing alone. Adopting risk-based follow-up algorithms including p16/Ki-67 triage for women with ASCUS or LSIL and colposcopy referral for women with HSIL or p16/Ki-67-positivity can reduce overtreatment. In the base-case analysis (31-43% screening adherence in women age 20-59 years), the IHBRs were 19 (5-yearly cytology+p16/Ki-67-triage), 31 (3-yearly cytology+p16/Ki-67-triage), 40 (3-yearly HPV+cytology cotesting), 45 (2-yearly HPV+ cytology cotesting), 131 (annual cytology +p16/Ki-67-triage), and 355 (annual p16/Ki-67 testing alone) unnecessary conizations per prevented cancer death. In populations with screening adherence below 40% biennial HPV+cytology cotesting may be performed. Depending on screening adherence rates, the screening interval may be extended to 3 years (40%-60% adherence) or to 5 years ($\geq 60\%$ adherence). The age for screening initiation could be extended from 18 to 24 years without significant loss in effectiveness, but with reduced overtreatments.

Conclusion

Based on our benefit-harm analysis, HPV-based screening in women at age 30 years and cytology in younger women at screening intervals of at least 2 years incorporating a risk-based follow-up algorithm can be recommended for the Austrian screening setting.

OC 14-16

AN ANALYTICAL QUALITY ASSESSMENT PROGRAMME FOR PRIMARY HRHPV SCREENING IN THE NETHERLANDS

A. Van Loon¹, W. Rodenburg¹, E. Brouwer², N. Van Der Veen²

¹National Institute for Public Health and the Environment, Centre for Health Protection, Bilthoven, the Netherlands (Netherlands), ²National Institute for Public Health and the Environment, Centre for Population Screening, Bilthoven, the Netherlands (Netherlands)

Background / Objectives

Development of an analytical quality assessment programme to assure reliable, consistent, high-quality laboratory results in the Dutch national hrHPV-based cervical cancer screening programme.

Methods

Five laboratories will be selected to carry out the renewed primary hrHPV based cervical screening programme in the Netherlands. All will use the same clinically validated hrHPV test system. Both clinically (pap-smear) and self-sampled material will be tested within the same workflow. The laboratories will all use the same QC reagents, and will report QC results in (nearly) real-time to a central database, which will allow monitoring of performance of the laboratories in a timely and fully transparent way.

In accordance with ISO 15189: 2012 Medical Laboratories – Requirements for quality and Competence, the programme for quality control of the analytical performance of hrHPV-testing in the laboratory will be based on the following three elements:

1. A programme for verification and acceptance testing of equipment upon installation, repair or major service, and of (new lots of) critical reagents and critical consumables.
2. A run control programme with a manufacturer-independent control sample in each hrHPV run to monitor day-to-day test variation, lot-to-lot performance of test kits, and operator variation, and will assist in identifying increased random or systematic error.
3. Interlaboratory comparisons such as external quality control programme with proficiency panels, some of which will be specific to the Dutch programme and some being part of a larger international programme.

As homogeneity and long term stability are essential requirement for these QC materials, control materials will consist of carefully selected dilutions of cell lines incorporating (parts) of the genome of hrHPV's

Results

A quality assessment programme that enables to monitor the quality of the analytical performance of the participating laboratories, to identify emerging problems and to take corrective action.

Conclusion

A strong analytical quality assessment programme is an essential part of the Dutch hrHPV screening programme for the prevention of cervical cancer. The implementation of the proposed quality assessment programme will enable to closely monitor the analytical performance of the participating laboratories in a timely and transparent manner.

OC 14-17

HPV self-sampling response rate in randomised study among Slovenian non-responders to the organised cervical cancer screening program

U. Ivanus¹, M. Primic Zakelj¹, T. Jerman¹, M. Florjancic¹, M. Kuster¹, I. Takac², A. Repse Fokter³

¹Cervical cancer screening programme and register ZORA, Epidemiology and cancer registries, Institute of Oncology Ljubljana (Slovenia), ²Division of Gynaecology and Perinatology, University Clinical Centre Maribor (Slovenia), ³Department of Cytopathology, Hospital General Celje (Slovenia)

Background / Objectives

A randomised controlled HPV self-sampling study was implemented in Slovenia with the aim to increase participation of non-responders to the organised population based cervical cancer screening programme ZORA. The study is still ongoing; it is coordinated by the national coordination office of programme ZORA and financed by Ministry of Health and Slovenian Research Agency (No. L3-5512). The objective of this analysis is to assess the acceptance of HPV self-sampling among Slovenian non-responders and association of response rate with a type of self-sampling device.

Methods

Non-responders aged 30–64 were randomly sampled from the national screening registry. They were allocated to the control arm (C) and two intervention arms for HPV self-sampling at home: I1 (n = 14.400, opt-in) and I2 (n = 9.556, opt-out). Women in I2 were randomly allocated to three groups I2-Q (n = 3,284), I2-H (n = 3,284) and I2-D (n = 2.988). Women who did not opt-out for self-sampling received one of three self-sampling devices (testers): women in I2-Q received Qvintip® (tester Q, Aprovix AB, Uppsala, Sweden), women in I2-H received HerSwab™ (tester H, Eve Medical Inc, Toronto, Canada) and women in I2-D received Delphi Screener (tester D, Rovers Medical Devices, Netherlands). In I1 only tester Q was used.

Results

Intention to screen response rate (no. of self-taken samples/no. women randomised with the intention to screen) and per-protocol response rate (no. of self-taken samples/no. of women who received tester per protocol) in intervention arms I1 and

I2 will be analysed. Multivariate logistic regression in SPSS 16.0 will be used with the type of tester and age of women as predictors for the response of women in I2.

Conclusion

HPV self-sampling is well accepted by Slovenian non-responders. The tester used for self-sampling can be a significant predictor of the response rate regardless the age of the women. To evaluate the clinical significance of these results the additional response that will include women without self-taken sample but with a Pap smear (after they were invited for self-sampling) will be assessed as well as the high-grade lesions detection rate.

OC 15-01

CONDYLOMATOSIS RECURRENCE AFTER SURGICAL TREATMENT: HPV QUADRIVALENT VACCINATION COULD REDUCE CLINICAL RELAPSE?

A. Ghelardi, P. Bay, A. Tonetti, A.F. Ragusa

Azienda USL Toscana nord ovest (Italy)

Background / Objectives

Anogenital warts (GW) are the most common HPV related disease. The GW incidence is more than 200 per 100.000 women, reaching about 8 per 1000 in women aged 20-25 years (1,2). Highly contagious, with elevated recurrence rates after treatment, GW are a relevant psychological and social problem (3). Although not associated to increased mortality GW are a social problem also in terms of costs, treatment is expensive, estimated cost over 800 USD per incident case in the United States (4). The major clinical problem of GW is the disease recurrence management, that is define as reappearance of GW within 12 months after complete clearance of the lesions. GW relapse, although related to type of surgical treatment and host's immuno-response, is around 50% (5). The aim of the study is to determine if HPV-vaccination post surgical treatment for GW can decrease the rate of disease recurrence.

Methods

We analysed the incidence of GW in women treated for cervical intraepithelial neoplasia. From a total of 398 enrolled patients we present data of women undergoing at least 6-months follow-up period (324 patients). We found 64 condylomatosis between the 324 patients (19%). All the patients were enrolled in a study for HPV quadrivalent vaccination after surgical treatment. 162 patients were enrolled in the vaccination arm (V-group) and 162 patients in the control unvaccinated group (C-group). Before treatment V-group GW incidence was 35/162 vs 29/162 of C-group. At 6 months follow-up visit, after treatment and vaccination, recurrence rates were analyzed into the two groups and statistical analysis by Pearson's chi squared test was performed.

Results

At 6 months follow-up visit 14 out of 29 patients in C-group developed a GW recurrence (47%) versus 5 of 35 vaccinated women recurred (14%). The rate of

recurrence was significantly higher in the unvaccinated group, with a $p=0.0053$ by Pearson's chi squared test.

Conclusion

According to our on-going studies and clinical findings from previous retrospective data, HPV-vaccination after treatment for GW may be useful in preventing recurrence of the disease. Vaccination could be useful in reducing social direct cost and psychological problems related to GW management.

References

- 1) Systematic review of incidence and prevalence of genital warts Patel H,Wagner M, Singhal P, Kothari S. BMC Infect Dis. 2013 Jan 25;13:39.
- 2) Rate and predictors of new genital warts claims and genital warts-related healthcare utilization among privately insured patients in the United States. Koshiol JE et al. Sex. Transm Dis 2004, 31(12): 748-752.
- 3) Human papillomavirus-related psychosocial impact of patients with genital warts in China: a hospital-based cross-sectional study.Qi SZ, Wang SM, Shi JF, Wang QQ, Chen XS, Sun LJ, Liu A, Zhang N, Jiang N, Siva P, Xu XL,Qiao YL. BMC Public Health. 2014 Jul 21;14:739.
- 4) Estimates of the annual direct medical costs of the prevention and treatment of disease associated with human papillomavirus in the United States.Chesson HW, Ekwueme DU, Saraiya M, Watson M, Lowy DR, Markowitz LE.Vaccine 2012 Sep 14;30(42):6016-9.
- 5) An evidence-based review of medical and surgical treatments of genital warts.Scheinfeld N, Lehman DS. Dermatol Online J. 2006 Mar 30;12(3):5.

OC 15-02

CLINICAL RELEVANCE AND TISSUE TROPISM OF THE MUPAPILLOMAVIRUS GENUS TYPES HPV1, HPV63 AND HPV204

A. Šterbenc¹, **L. Hošnjak**¹, **B.J. Kocjan**¹, **A.A. Giri**², **D. Chouhy**², **E.M. Bolatti**², **M. Poljak**¹

¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana (Slovenia), ²Virology Area, School of Biochemistry and Pharmaceutical Sciences, Rosario National University, Rosario (Argentina)

Background / Objectives

Mupapillomavirus (*Mu-PV*) genus currently consists of only 3 members: HPV1, HPV63 and HPV204. HPV1 and HPV63 were identified in 1980 and 1993, respectively, and are associated with the development of common warts. HPV204 is a novel *Mu-PV* type identified in 2014 with a yet undetermined clinical significance and tissue tropism (1). The aim of the study was to determine the clinical relevance and tissue tropism of HPV1, HPV63 and HPV204.

Methods

Quantitative HPV1/HPV63/HPV204 type-specific real-time PCR assays with a sensitivity of at least 10 viral copies/assay were developed and used to test a representative collection of various HPV-associated benign and malignant neoplasms of the skin and mucosa, clinically normal mucosal samples and eyebrow hair follicles (n=1,006). HPV1, HPV63 and HPV204 viral loads per single human cell were estimated for all tissue samples.

Results

HPV1 was detected in 2/110 (1.8%) of nasopharyngeal swab samples, 21/43 (48.8%) of common warts, 1/110 (0.9%) of eyebrow hair follicles and 3/40 (7.5%) of anogenital warts, HPV63 in 2/105 (1.9%) of oral mucosa swab samples, 3/110 (2.7%) of nasopharyngeal swab samples, 9/43 (20.9%) of common warts, 4/110 (3.6%) of eyebrow hair follicles and 1/110 (0.9%) of anal canal swabs, while HPV204 was detected only in 1/116 (0.9%) of cervical swab samples, 1/43 (2.3%) of common warts, 4/110 (3.6%) of anal canal swabs and 5/110 (4.5%) of penile surface swabs. HPV1 viral load ranged from 5.9×10^{-6} to 3.2×10^{-5} copies/cell in anogenital warts, 2.6×10^{-5} to 5.2×10^4 copies/cell in common warts and was 1.5×10^{-5} copies/cell in an eyebrow hair follicle. HPV63 viral loads ranged from 2.5×10^{-6} to 3.4×10^{-5}

copies/cell in eyebrow hair follicles and from 3.2×10^{-6} to 3.9×10^2 copies/cell in common warts. Viral load of HPV204 in a common wart was low (7.3×10^{-7} copies/cell).

Conclusion

Our study has expanded the current knowledge of tissue tropism of *Mu*-PV types. Although the prevalence of *Mu*-PV types is generally low (1.1%-2.7%), HPV1, HPV63 and HPV204 can be detected in virtually all types of benign cutaneous and mucosal samples, suggesting that all members of *Mu*-PV genus exhibit dual tissue tropism. On the contrary, none of the *Mu*-PV types were found in malignant neoplasms. HPV204 appears to cause only latent infections of the skin and mucosa, though further studies are needed to evaluate its potential role in the development of common warts.

References

(1) Kocjan BJ, Šterbenc A, Hošnjak L, Chouhy D, Bolatti E, Giri AA, Poljak M. Genome announcement: complete genome sequence of a novel Mupapillomavirus, HPV204. *Acta Dermatovenerol Alp Pannonica Adriat.* 2015;24(2):21-3.

OC 15-03

HUMAN PAPILOMA VIRUS (HPV) ASSOCIATED WITH BODY MASS INDEX (BMI) IN 4487 THAI WOMEN UNDER CERVICAL SCREENING PROGRAM.

U. Chatchotikawong¹, **N. Kantathavorn**¹, **S. Thaniya**², **T. Chantane**³, **S. Siriporn**³, **K. Waraphorn**³, **K. Sathiya**³, **T. Narongchai**², **K. Wasanai**¹

¹Gynecologic Oncology Unit, Chulabhorn Hospital, Bangkok (Thailand),

²Pathology Unit, Chulabhorn Hospital, Bangkok (Thailand), ³Chulabhorn Hospital (Thailand)

Background / Objectives

Thailand is a cervical carcinoma endemic area. Obesity, high BMI value, is known to be associated with many diseases including a high risk of cervical cancer death. In this work, a large cohort of Thai women was explored to determine the association between human papilloma virus (HPV) infection and body mass index (BMI).

Methods

HPV genotyping data from 4487 women aged from 20 to 70 year-old was collected and analyzed in a hospital-based cervical cancer screening program at Chulabhorn Hospital, Bangkok, Thailand. The kit of linear array HPV testing, capable of identifying 37 HPV types including 12 high-risk (HR), 8 probable high-risk (PR), and 17 low-risk (LR) types classified by oncogenic potentiality, was used to analyze HPV genotyping results.

Results

From the study, 15.1%, 6.4%, 3.5% and 8.4% of the cohort were found to have HPV infection, HR, PR, and LR, respectively. The cohort with BMI 25.00-29.99 kg/m² has significantly low Odds Ratio (OR) of 0.48, lower than that of the cohort with BMI < 18.5 kg/m², for high-risk HPV positive type at 95% confidence interval of 0.29-0.80. The same cohort has significantly high OR of 1.49 for HPV negative at 95% confidence interval of 1.04-2.13; significantly low OR of 0.67 for HPV positive at 95% confidence interval of 0.47-0.96. The cohort with BMI ≥ 30 kg/m² (obesity) is not found to be significantly associated with HPV infection (HPV negative, HPV positive, high-risk positive HPV, and low-risk HPV positive). However, the OR value is found to be significantly low of 0.25 for probable high-risk HPV with 95% confidence interval of 0.07-0.94.

Odds ratio (95% confidence interval) for HPV typing (N=4487); p<0.05*

	HPV negative	HPV positive	High-risk HPV positive	Probable-risk HPV positive	Low-risk HPV positive
BMI <18.5 kg/m ²	1.00	1.00	1.00	1.00	1.00
BMI 18.50-22.99 kg/m ²	1.12 (0.80-1.56)	0.90 (0.64-1.24)	0.88(0.56-1.39)	1.14 (0.56-2.30)	0.90 (0.59-1.38)
BMI 23.00-24.99 kg/m ²	1.06 (0.75-1.53)	0.93 (0.65-1.34)	0.60 (0.36-1.01)	1.46 (0.70-3.06)	1.01 (0.64-1.60)
BMI 25.00-29.99 kg/m ²	1.49 (1.04-2.13)*	0.67 (0.47-0.96)*	0.48 (0.29-0.80)*	0.90 (0.42-1.90)	0.75 (0.47-1.18)
BMI ≥30.00 kg/m ²	1.49 (0.96-2.31)	0.67 (0.43-1.04)	0.63 (0.34-1.16)	0.25 (0.07-0.94)*	0.73 (0.41-1.28)

Conclusion

Overweight women (BMI 25.00-29.99 kg/m²) have lower HPV high-risk positive infection than other BMI groups. High-risk HPV infection was not found to be significant in BMI of <18.50, 18.50-22.99, and 23.00-24.99 kg/m². The low rate of HPV infection in all women in the study group is due to the widely adopted HPV vaccination for primary prevention of cervical cancer in Thailand.

OC 15-04

CERVICAL CANCER SCREENING IN THE NETHERLANDS: DETERMINATION OF HPV PREVALENCE USING THREE DIFFERENT SYSTEMS

C.J. Huijsmans¹, W.R. Geurts-Giele², C. Leeijen¹, E. Hazenberg¹, J. Van Beek³, J.C. Van Der Linden¹, A.J. Van Den Brule¹

¹Pathologie-DNA, location Jeroen Bosch Hospital, 's-Hertogenbosch (Netherlands), ²Pathologie-DNA, location Jeroen Bosch Hospital, 's-Hertogenbosch and Erasmus MC Cancer Institute, Department of Pathology, Rotterdam (Netherlands), ³Pathologie-DNA, location Rijnstate Hospital, Arnhem (Netherlands)

Background / Objectives

Primary high risk (hr)HPV screening will be introduced in The Netherlands during the 1st quarter of 2017. Our aim was to determine the hrHPV prevalence in a cohort of women representative for the Dutch population based screening program.

Methods

A total of 11,802 residual PreservCyt cervical samples from the Dutch population based cytology screening program were rendered anonymous, randomized and tested for hrHPV using 3 completely automated HPV detection systems: Qiagen Hybrid Capture 2 (HC2, signal amplification), Roche Cobas® 4800 (DNA amplification) and Hologic Aptima (RNA amplification). To determine the agreement between results generated using the different assays, pair wise comparison of the systems was performed by determining kappa coefficients.

Results

The selected samples were representative for the population based screening program with respect to age distribution and cytology classification. HrHPV prevalences found were: 8.1% for HC2, 8.0% for Cobas® 4800 and 7.6% for Aptima, resulting in a mean hrHPV prevalence of $7.9 \pm 0.3\%$. Therefore, independently of the assay used, the hrHPV prevalence is significantly higher than the previously reported 4-5% using the GP5+/6+PCR-EIA (POBASCAM) and HC2 (VUSA-Screen) hrHPV tests. As expected, a clear age dependency was found, with an hrHPV prevalence ranging from $18.6 \pm 1.1\%$ in women 29-33 years of age to $3.9 \pm 0.3\%$ in women 59-63 years of age. Also for severity of cytology a correlation with hrHPV prevalence was observed, ranging from $5.4 \pm 0.3\%$ in normal cytology to $92.2 \pm 3.1\%$ in severe

dysplasia. Kappa coefficients of 0.77, 0.71 and 0.72 (HC2 vs Cobas® 4800, Cobas® 4800 vs Aptima and Aptima vs HC2, respectively) indicated substantial agreement between the results generated.

Conclusion

In contrast to the report of the Dutch Health Council, a higher hrHPV prevalence of $7.9\pm 0.3\%$ was found in this population based screening cohort using the complete hrHPV detection systems from Qiagen HC2, Roche Cobas® 4800, and Hologic Aptima, which has consequences for the cost-effectiveness of the Dutch screening program. Additionally, based on the kappa coefficients, agreement of results between the 3 hrHPV testing solutions was substantial.

OC 15-05

Identification HPV Integration Sites of CIN and Cervical Cancer Patients in Shanghai Women

F. Li

Department of Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine (China)

Background / Objectives

Abstract

[Background] Virus integration into the host genome is one of the critical steps that lead to the progression of the precancerous lesion into cancer. This study aims to identify integration sites of two High Risk-HPV types (16 and 52) in women with cervical intraepithelial neoplasia(CIN) and cervical cancer in Shanghai and to study the relationship between integration and occurrence of cervical cancer. This is the first time HPV52 integration sites were studied.

Methods

[Methods] HPV DNA integrated status of totally 19 clinical samples including 13 single HPV16 infective and 6 single HPV52 infective samples with CIN or cervical squamous cell carcinoma (SCC) were detected by ligation-mediated PCR (DIPS-PCR) and DNA sequences. In total, 12 published nested primers were used in PCR for HPV16 and nine new nested-PCR primer sets were specifically designed for HPV52 viral-cellular junction analysis. All the results were analyzed by NCBI BLAST and NCBI-Map Viewer.

Results

[Result] HPV16 integration rates in SCC samples is 60% (3/5), in CINII-III is 60% (3/5) and in CIN I is 0%(0/3). Ten integration events were found in human genomes from HPV16 infected samples, including 3 SCC and 3 CINII-III. Single integration site was found in all three samples. Each of two samples has two integration sites.. And three integration sites were found in only one sample. HPV DNA integrated into SCAI, NAALADL2, KALRN and SNX25 gene distributed in human chromosomes 3, 4, 9, 17 and 22 respectively. Both chromosome 3 and 22 were integrated twice. And 100% of the integration sites located in the intron of the genes. In the eight

integration sites identified, seven (87.5%) occurred near the common fragile sites of human genomes. Our results also shown that there were thirteen disruption sites in HPV genomes, in which six of them were happened at HPV16 E1, two at E2, two at L1, one at L2, one at E5 gene, and one happened at at E1 gene diffused with HPV16 E2 gene. The integration sites of HPV52 were also identified successfully.

Conclusion

[Conclusion]Our observations suggest that integration is a late events in HPV16 infection patients with CIN. All the HPV16 integration sites are in the intron area of human genome, two of them are cancer associated genes. These integration coexist with host genomic abnormal including translocation, breakage, recombination, deletions and chromosomal translocations. It is possible that HPV16 DNA integration plays a key role in tumor development.

References

References:

- [1] Li J, Kang LN, Qiao YL. Review of the cervical cancer disease burden in mainland China. *Asian Pac J Cancer Prev* 2011;12: 1149-53.
- [2] Li H, Yang Y, Zhang R, Cai Y, Yang X, Wang Z, Li Y, Cheng X, Ye X, Xiang Y, Zhu B. Preferential sites for the integration and disruption of human papillomavirus 16 in cervical lesions. *J Clin Virol* 2013;56: 342-7.
- [3] De Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: A retrospective cross-sectional worldwide study. *Lancet Oncol* 2010; 11:1048–1056.
- [4] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348: 518-27.
- [5] Bao YP, Li N, Smith JS, Qiao YL. Human papillomavirus type distribution in women from Asia: a meta-analysis. *Int J Gynecol Cancer* 2008;18: 71-9.
- [6] Natphopsuk S, Settheetham-Ishida W, Pientong C, Sinawat S, Yuenyao P, Ishida T, Settheetham D. Human papillomavirus genotypes and cervical cancer in northeast Thailand. *Asian Pac J Cancer Prev* 2013;14: 6961-4.
- [7] Lee EH, Um TH, Chi HS, Hong YJ, Cha YJ. Prevalence and distribution of human papillomavirus infection in Korean women as determined by restriction fragment mass polymorphism assay. *J Korean Med Sci* 2012;27: 1091-7.
- [8] Quek SC, Lim BK, Domingo E, Soon R, Park JS, Vu TN, Tay EH, Le QT, Kim YT, Vu BQ, Cao NT, Limson G, Pham VT, Molijn A, Ramakrishnan G, Chen J.

Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical intraepithelial neoplasia across 5 countries in Asia. *Int J Gynecol Cancer* 2013;23: 148-56.

[9] Frame FM, Rogoff HA, Pickering MT, Cress WD, Kowalik TF. E2F1 induces MRN foci formation and a cell cycle checkpoint response in human fibroblasts. *Oncogene* 2006;25: 3258-66.

[10] Pickering MT, Kowalik TF. Rb inactivation leads to E2F1-mediated DNA double-strand break accumulation. *Oncogene* 2006;25: 746-55.

[11] Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B. Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell* 2011;145: 435-46.

[12] Schwarz E, Freese UK, Gissmann L, Mayer W, Roggenbuck B, Stremlau A, Zur HH. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 1985;314: 111-4.

[13] Wells SI, Francis DA, Karpova AY, Dowhanick JJ, Benson JD, Howley PM. Papillomavirus E2 induces senescence in HPV-positive cells via pRB- and p21(CIP)-dependent pathways. *EMBO J* 2000;19: 5762-71.

[14] Sastre-Garau X, Favre M, Couturier J, Orth G. Distinct patterns of alteration of myc genes associated with integration of human papillomavirus type 16 or type 45 DNA in two genital tumours. *J Gen Virol* 2000;81: 1983-93.

[15] Pett MR, Alazawi WO, Roberts I, Dowen S, Smith DI, Stanley MA, Coleman N. Acquisition of high-level chromosomal instability is associated with integration of human papillomavirus type 16 in cervical keratinocytes. *Cancer Res* 2004;64: 1359-68.

[16] Winder DM, Pett MR, Foster N, Shivji MK, Herdman MT, Stanley MA, Venkitaraman AR, Coleman N. An increase in DNA double-strand breaks, induced by Ku70 depletion, is associated with human papillomavirus 16 episome loss and de novo viral integration events. *J Pathol* 2007;213: 27-34.

[17] Cullen AP, Reid R, Campion M, Lorincz AT. Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasm. *J Virol* 1991;65: 606-12.

[18] Luft F, Klaes R, Nees M, Durst M, Heilmann V, Melsheimer P, von Knebel DM. Detection of integrated papillomavirus sequences by ligation-mediated PCR (DIPS-PCR) and molecular characterization in cervical cancer cells. *Int J Cancer* 2001;92: 9-17.

[19] Peter M, Stransky N, Couturier J, Hupe P, Barillot E, de Cremoux P, Cottu P, Radvanyi F, Sastre-Garau X. Frequent genomic structural alterations at HPV insertion sites in cervical carcinoma. *J Pathol* 2010;221: 320-30.

- [20] Matovina M, Sabol I, Grubisic G, Gasperov NM, Grce M. Identification of human papillomavirus type 16 integration sites in high-grade precancerous cervical lesions. *Gynecol Oncol* 2009;113: 120-7.
- [21] Wentzensen N, Ridder R, Klaes R, Vinokurova S, Schaefer U, Doeberitz M. Characterization of viral-cellular fusion transcripts in a large series of HPV16 and 18 positive anogenital lesions. *Oncogene* 2002;21: 419-26.
- [22] Wentzensen N, Vinokurova S, von Knebel DM. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res* 2004;64: 3878-84.
- [23] Ferber MJ, Thorland EC, Brink AA, Rapp AK, Phillips LA, McGovern R, Gostout BS, Cheung TH, Chung TK, Fu WY, Smith DI. Preferential integration of human papillomavirus type 18 near the c-myc locus in cervical carcinoma. *Oncogene* 2003;22: 7233-42.
- [24] Thorland EC, Myers SL, Gostout BS, Smith DI. Common fragile sites are preferential targets for HPV16 integrations in cervical tumors. *Oncogene* 2003;22: 1225-37.
- [25] Ziegert C, Wentzensen N, Vinokurova S, Kisseljov F, Einenkel J, Hoeckel M, von Knebel DM. A comprehensive analysis of HPV integration loci in anogenital lesions combining transcript and genome-based amplification techniques. *Oncogene* 2003;22: 3977-84.
- [26] Peter M, Rosty C, Couturier J, Radvanyi F, Teshima H, Sastre-Garau X. MYC activation associated with the integration of HPV DNA at the MYC locus in genital tumors. *Oncogene* 2006;25: 5985-93.
- [27] Kraus I, Driesch C, Vinokurova S, Hovig E, Schneider A, von Knebel DM, Durst M. The majority of viral-cellular fusion transcripts in cervical carcinomas cotranscribe cellular sequences of known or predicted genes. *Cancer Res* 2008;68: 2514-22.
- [28] Dall KL, Scarpini CG, Roberts I, Winder DM, Stanley MA, Muralidhar B, Herdman MT, Pett MR, Coleman N. Characterization of naturally occurring HPV16 integration sites isolated from cervical keratinocytes under noncompetitive conditions. *Cancer Res* 2008;68: 8249-59.
- [29] Matovina M, Sabol I, Grubisic G, Gasperov NM, Grce M. Identification of human papillomavirus type 16 integration sites in high-grade precancerous cervical lesions. *Gynecol Oncol* 2009;113: 120-7.
- [30] Zhang C, Zhang S, Zhang Z, He J, Xu Y, Liu S. ROCK has a crucial role in regulating prostate tumor growth through interaction with c-Myc. *Oncogene* 2013.
- [31] Feng B, Li K, Zhong H, Ren G, Wang H, Shang Y, Bai M, Liang J, Wang X, Fan D. RhoE Promotes Metastasis in Gastric Cancer through a Mechanism Dependent on Enhanced Expression of CXCR4. *PLoS One* 2013;8: e81709.

- [32] Dziuba N, Ferguson MR, O'Brien WA, Sanchez A, Prussia AJ, McDonald NJ, Friedrich BM, Li G, Shaw MW, Sheng J, Hodge TW, Rubin DH, Murray JL. Identification of cellular proteins required for replication of human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* 2012;28: 1329-39.
- [33] Schwarz E, Freese UK, Gissmann L, Mayer W, Roggenbuck B, Stremlau A, Zur HH. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 1985;314: 111-4.
- [34] von Knebel DM, Bauknecht T, Bartsch D, Zur HH. Influence of chromosomal integration on glucocorticoid-regulated transcription of growth-stimulating papillomavirus genes E6 and E7 in cervical carcinoma cells. *Proc Natl Acad Sci U S A* 1991;88: 1411-5.
- [35] Jeon S, Allen-Hoffmann BL, Lambert PF. Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *J Virol* 1995;69: 2989-97.
- [36] Schmitz M, Driesch C, Jansen L, Runnebaum IB, Durst M. Non-random integration of the HPV genome in cervical cancer. *PLoS One* 2012;7: e39632.
- [37] Iliopoulos D, Guler G, Han SY, Druck T, Ottey M, McCorkell KA, Huebner K. Roles of FHIT and WWOX fragile genes in cancer. *Cancer Lett* 2006;232: 27-36.
- [38] Ozeri-Galai E, Bester AC, Kerem B. The complex basis underlying common fragile site instability in cancer. *Trends Genet* 2012;28: 295-302.
- [39] Matovina M, Sabol I, Grubisic G, Gasperov NM, Grce M. Identification of human papillomavirus type 16 integration sites in high-grade precancerous cervical lesions. *Gynecol Oncol* 2009;113: 120-7.
- [40] Wentzensen N, Vinokurova S, von Knebel DM. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res* 2004;64: 3878-84.
- [41] Lukusa T, Fryns JP. Human chromosome fragility. *Biochim Biophys Acta* 2008;1779: 3-16.
- [42] Schwartz M, Zlotorynski E, Kerem B. The molecular basis of common and rare fragile sites. *Cancer Lett* 2006;232: 13-26.
- [43] Peter M, Rosty C, Couturier J, Radvanyi F, Teshima H, Sastre-Garau X. MYC activation associated with the integration of HPV DNA at the MYC locus in genital tumors. *Oncogene* 2006;25: 5985-93.
- [44] Klimov E, Vinokourova S, Mojsjak E, Rakhmanaliev E, Kobseva V, Laimins L, Kisseljov F, Sulimova G. Human papilloma viruses and cervical tumours: mapping of integration sites and analysis of adjacent cellular sequences. *BMC Cancer* 2002;2: 24.

- [45] Brandt DT, Baarlink C, Kitzing TM, Kremmer E, Ivaska J, Nollau P, Grosse R. SCAI acts as a suppressor of cancer cell invasion through the transcriptional control of beta1-integrin. *Nat Cell Biol* 2009;11: 557-68.
- [46] Brauchle M, Yao Z, Arora R, Thigale S, Clay I, Inverardi B, Fletcher J, Taslimi P, Acker MG, Gerrits B, Voshol J, Bauer A, Schubeler D, Bouwmeester T, Ruffner H. Protein complex interactor analysis and differential activity of KDM3 subfamily members towards H3K9 methylation. *PLoS One* 2013;8: e60549.
- [47] Fintha A, Gasparics A, Fang L, Erdei Z, Hamar P, Mozes MM, Kokeny G, Rosivall L, Sebe A. Characterization and role of SCAI during renal fibrosis and epithelial-to-mesenchymal transition. *Am J Pathol* 2013;182: 388-400.
- [48] Tonkin ET, Smith M, Eichhorn P, Jones S, Imamwerdi B, Lindsay S, Jackson M, Wang TJ, Ireland M, Burn J, Krantz ID, Carr P, Strachan T. A giant novel gene undergoing extensive alternative splicing is severed by a Cornelia de Lange-associated translocation breakpoint at 3q26.3. *Hum Genet* 2004;115: 139-48.
- [49] Brumby AM, Goulding KR, Schlosser T, Loi S, Galea R, Khoo P, Bolden JE, Aigaki T, Humbert PO, Richardson HE. Identification of novel Ras-cooperating oncogenes in *Drosophila melanogaster*: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. *Genetics* 2011;188: 105-25.
- [50] Du Y, Zou Y, Yu W, Shi R, Zhang M, Yang W, Duan J, Deng Y, Wang X, Lu Y. Expression pattern of sorting Nexin 25 in temporal lobe epilepsy: a study on patients and pilocarpine-induced rats. *Brain Res* 2013;1509: 79-85.
- [51] Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B. Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell* 2011;145: 435-46.
- [52] You J. Papillomavirus interaction with cellular chromatin. *Biochim Biophys Acta* 2010;1799: 192-9.
- [53] Dey A, Chitsaz F, Abbasi A, Misteli T, Ozato K. The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. *Proc Natl Acad Sci U S A* 2003;100: 8758-63.
- [54] Angulo M, Carvajal-Rodriguez A. Evidence of recombination within human alpha-papillomavirus. *Virology* 2007;4: 33.
- [55] Chen Z, Schiffman M, Herrero R, Desalle R, Anastos K, Segondy M, Sahasrabudhe VV, Gravitt PE, Hsing AW, Burk RD. Evolution and taxonomic classification of human papillomavirus 16 (HPV16)-related variant genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67. *PLoS One* 2011;6: e20183.
- [56] Chen Z, Terai M, Fu L, Herrero R, DeSalle R, Burk RD. Diversifying selection in human papillomavirus type 16 lineages based on complete genome analyses. *J Virol* 2005;79: 7014-23.

[57] Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, Hildesheim A, Desalle R, Befano B, Yu K, Safaeian M, Sherman ME, Morales J, Guillen D, Alfaro M, Hutchinson M, Solomon D, Castle PE, Burk RD. A population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia. *Cancer Res* 2010;70: 3159-69.

[58] Ganguly N. Human papillomavirus-16 E5 protein: oncogenic role and therapeutic value. *Cell Oncol (Dordr)* 2012;35: 67-76.

OC 15-06

BIOTINYLATED-TYRAMIDE-BASED IN SITU HYBRIDIZATION SIGNAL PATTERNS IN THE DETECTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS IN CERVICAL SAMPLES FROM WOMEN IN BAGHDAD PROVINCE

T.J.M. Al-Khishali

Department of Anatomy/ College of Medicine/ University of Baghdad (Iraq)

Background / Objectives

BACKGROUND: Despite the fact that in situ hybridization (ISH) has been extensively studied, there have been limited reports of the usefulness of this technique in malignant transformation and HPV-DNA integration in relation to histological findings (24. De Marchi Triglia R. 2009, 26. Ming Guo. 2008, 31. Zappacosta R 2013)

OBJECTIVE: To assess the usefulness of HPV genotyping using in situ hybridization ISH technique to identify episomal and punctate signal pattern and to verify whether a punctate pattern could be used as a progression marker of CIN

Methods

METHODS: The study period was between June 2013 through July 2014. HPV status was determined in 40 ever-married women (25 - 53 years age) using in situ hybridization, 20 women (group I) with healthy looking cervixes and normal Pap smear, and 20 women (group II) with abnormal cervixes and abnormal Pap smears. A third group, (group III), 20 women with squamous cell carcinoma. Pap smears were collected, followed by colposcopic guided biopsy. HPV was tested on all specimens by in situ hybridization (ISH) using the broad spectrum HPV probe recognizing HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 compared with conventional hematoxylin and eosin H&E stain

Results

RESULTS: 4 of the 20 women (group I) showed mild positive ISH test results with diffuse nuclear staining. Group II results; mild positive reaction with diffuse nuclear signal pattern was noticed in 4 (20%) of the cervical specimens. Moderate positive

reaction with diffuse signal pattern of the nucleus was observed in 8 (40%) of the specimens. The remaining 8 (40%) of the specimens showed both diffuse and punctate signals in the nuclei.

Conclusion

CONCLUSION: ISH is a sensitive, easy to handle method for HPV-detection in cervical squamous intraepithelial lesions and cancers, suitable for routine pathology, helps to distinguish episomal from integrated HPV, and hence, prognosis of the lesion.

References

References

24. De Marchi Triglia R., Metze K, Zeferino LC, Lucci De Angelo Andrade LA: HPV in situ hybridization signal patterns as a marker for cervical intraepithelial neoplasia progression. *Gynecol Oncol.* 2009;112:114–118. (Abstract)

26. Ming Guo, Yun Gong, Michael Deavers, Elvio G. Silva, Yee Jee Jan, David E. Cogdell, Rajyalashmi Luthra, E. Lin, Hung Cheng Lai, Wei Zhang, and Nour Sneige, Evaluation of a commercialized In situ hybridization assay for detecting human papillomavirus DNA in tissue specimens from patients with cervical intraepithelial neoplasia and Cervical Carcinoma, *Journal of Clinical Microbiology*, Jan. 2008, p. 274–280 Vol. 46, No. 10095-1137

31. Zappacosta R, Antonella Colasante, Patrizia Viola, Tommaso D'Antuono, Giuseppe Lattanzio, Serena Capanna, Daniela Maria Pia Gatta, and Sandra Rosini: Chromogenic in situ hybridization and p16/Ki67 dual staining on formalin-fixed paraffin-embedded cervical specimens: correlation with HPV-DNA test, E6/E7 mRNA test, and potential clinical applications. *BioMed Research International*. Volume 2013, Article ID 453606, 11 pages

Key words: Cervical intraepithelial neoplasia CIN, in situ hybridization ISH, human papillomavirus HPV

OC 15-07

ANALYSIS OF HUMAN PAPILLOMAVIRUS TYPE-16 AND -18 LINEAGES IN IRANIAN WOMEN BASED ON LONG CONTROL GENE REGION

S.A. Nadji¹, M. Mobini Kesheh², M. Barazandeh², S. Sharif Kashani², S. Mirab Samiee³, A. Motlagh⁴

¹Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran. (Iran, islamic republic of), ²National HPV reference Laboratory, Tehran, Iran. (Iran, islamic republic of), ³Reference Health Laboratory, Ministry of Health and Medical Education, Iran. (Iran, islamic republic of), ⁴Cancer Office, Non-Communicable Diseases, Deputy of Health, Ministry of Health and Medical Education, Tehran, Iran (Iran, islamic republic of)

Background / Objectives

Both HPV-16 and HPV-18 play a prominent role in the development of cervical cancer and it was suggested that some variants of HPV-16 and HPV-18 might confer differential risk of cervical disease. The genetic lineages of HPV16 and 18 are not known in Iran. The purpose of this study was to analyze variations of HPV-16 and -18 lineages within the LCR region.

Methods

A total of 2756 samples; including 2638 Cervical ThinPrep and 118 vaginal swabs from Iranian females of 12 provinces, were subjected to HPV screening and then genotyping. Totally 56 samples became positive for HPV-16 (41 samples) and HPV-18 (15 samples). To analyze the HPV lineages, a partial nucleotide sequence of LCR region was sequenced and phylogenetic and SNP analysis were performed using MEGA 5.05 software and HPV genome references.

Results

Sequence analysis of HPV-16 showed that sub-lineage NA1 are the major variant (81.6%, n=31). After NA, sub-lineage EP1 (15.8%, n=6) and the only African variant (AFR2a) were followed. 10 new SNPs were observed in studied HPV-16 sequences. Two new SNPs, T7436G and C7782T, were observed in most of isolates under sub-lineage NA. Eu lineage of HPV-18 is the most prevalent variant in Iran (92.3%, n-12), that followed by As-Am. No AF variant of HPV-18 was found in our study.

Conclusion

We now report the distribution of HPV16 and -18 variants in Iran. Despite other Asian countries, Non-Asian HPV-16 and HPV-18 variants are prevalent in Iran. A possible reason of low incidence of cervical cancer in Iranian women could be explained by this observation.

EUROGIN 2016 ABSTRACTS

Part III POSTERS

P1 Cervical neoplasia.....	P.2
P2 Colposcopy and management.....	P.22
P3 Conventional therapies.....	P.26
P4 Cytology.....	P.30
P5 Diagnostic procedures.....	P.43
P6 Economics and modelling.....	P.49
P7 Epidemiology and natural history.....	P.51
P8 Genomics.....	P.86
P9 Health education.....	P.92
P10 HPV oropharynx/ Head and neck cancer.....	P.97
P11 HPV prophylactic vaccines.....	P.106
P12 HPV testing	P.126
P.13 Immunology.....	P.179
P 14 Low resource settings.....	P.181
P15 Molecular markers.....	P.183
P16 New technologies.....	P.193
P17 Screening methods.....	P.200
P18 Sexually transmitted diseases and HPV infection.....	P.223
P19 Vulvar diseases and neoplasia.....	P.229
P20 HPV therapeutic vaccines.....	P.236

P01-01

Prognostic factors affecting survival in cervical cancer patients with recurrent parenchymal lung lesions without other organ involvement

L. Yoo-Young, K. Byoung-Gie, B. Duk-Soo, L. Jeong-Won, C. Chel Hun, K. Tae-Joong

Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea (Korea, republic of)

Background / Objectives

Distant recurrence from solid tumors shows dismal prognosis. However, recurrence limited to lungs is reported to have relatively long term disease free survival. We assessed the prognostic factors affecting survival outcomes and feasibility of pulmonary metastasectomy in cervical cancer patients who had recurrent disease limited to lungs.

Methods

We retrospectively enrolled the patients treated at Samsung Medical Center from 1996 to 2010. Among 2222 patients with cervical cancer, 39 patients who had recurrent cervical cancer limited to lung parenchyma were finally included for the analysis. Age, number of metastatic lesions, metastasectomy, histology, and treatment free interval were included in the Cox model and subsequent recurrence pattern was analyzed.

Results

The median follow-up was 56.7 months (15-121 months) and median overall survival after recurrence was 26.5 months (1-70 months) with 5-year overall survival of 37.1%. The median time interval from the end of previous treatment and diagnosis of the recurrence was 31.3 months (7-67 months). Nineteen of 39 patients (48.7%) had metastasectomy for lung lesions and adjuvant therapy was added in 84.2% (16/19) of these patients. On multivariate analysis, the number of metastatic lesions (1, 2, 3 vs. ≥ 4 ; HR, 8.97; 95% CI, 1.174-68.58; $p=0.034$) were statistically significant prognostic factors for overall survival.

Conclusion

Recurrent cervical cancer limited to the lungs is associated with long term overall survival with multidisciplinary treatment approach including surgery and systemic chemotherapy when there are ≤ 3 metastases.

References

- 1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127: 2893-2917, 2010.
- 2 Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, Heintz AP, Ngan HY and Pecorelli S: Carcinoma of the cervix uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet* 95 Suppl 1: S43-103, 2006.
- 3 Tellis CJ and Beechler CR: Pulmonary metastasis of carcinoma of the cervix: a retrospective study. *Cancer* 49: 1705-1709, 1982.
- 4 Sostman HD and Matthay RA: Thoracic metastases from cervical carcinoma: current status. *Invest Radiol* 15: 113-119, 1980.
- 5 Panek G, Gawrychowski K, Sobiczewski P, Derlatka P, Danska-Bidzinska A, Gmyrek L and Bidzinski M: Results of chemotherapy for pulmonary metastases of carcinoma of the cervix in patients after primary surgical and radiotherapeutic management. *Int J Gynecol Cancer* 17: 1056-1061, 2007.
- 6 Clavero JM, Deschamps C, Cassivi SD, Allen MS, Nichols FC, 3rd, Barrette BA, Larson DR and Pairolero PC: Gynecologic cancers: factors affecting survival after pulmonary metastasectomy. *Ann Thorac Surg* 81: 2004-2007, 2006.
- 7 Inoue M, Ohta M, Iuchi K, Matsumura A, Ideguchi K, Yasumitsu T, Nakagawa K, Fukuhara K, Maeda H, Takeda S, Minami M, Ohno Y and Matsuda H: Benefits of surgery for patients with pulmonary metastases from colorectal carcinoma. *Ann Thorac Surg* 78: 238-244, 2004.
- 8 Yamamoto K, Yoshikawa H, Shiromizu K, Saito T, Kuzuya K, Tsunematsu R and Kamura T: Pulmonary metastasectomy for uterine cervical cancer: a multivariate analysis. *Ann Thorac Surg* 77: 1179-1182, 2004.
- 9 Imachi M, Tsukamoto N, Matsuyama T and Nakano H: Peritoneal cytology in patients with carcinoma of the uterine cervix. *Gynecol Oncol* 26: 202-207, 1987.
- 10 Imachi M, Tsukamoto N, Matsuyama T and Nakano H: Pulmonary metastasis from carcinoma of the uterine cervix. *Gynecol Oncol* 33: 189-192, 1989.
- 11 Pastorino U, Buyse M, Friedel G, Ginsberg RJ, Girard P, Goldstraw P, Johnston M, McCormack P, Pass H and Putnam JB, Jr.: Long-term results of lung metastasectomy: prognostic analyses based on 5206 cases. *J Thorac Cardiovasc Surg* 113: 37-49, 1997.

- 12 Fuller AF, Jr., Scannell JG and Wilkins EW, Jr.: Pulmonary resection for metastases from gynecologic cancers: Massachusetts General Hospital experience, 1943-1982. *Gynecol Oncol* 22: 174-180, 1985.
- 13 Mountain CF, McMurtrey MJ and Hermes KE: Surgery for pulmonary metastasis: a 20-year experience. *Ann Thorac Surg* 38: 323-330, 1984.
- 14 McCormack P: Surgical resection of pulmonary metastases. *Semin Surg Oncol* 6: 297-302, 1990.
- 15 Martini N and McCormack PM: Evolution of the surgical management of pulmonary metastases. *Chest Surg Clin N Am* 8: 13-27, 1998.
- 16 Barter JF, Soong SJ, Hatch KD, Orr JW and Shingleton HM: Diagnosis and treatment of pulmonary metastases from cervical carcinoma. *Gynecol Oncol* 38: 347-351, 1990.
- 17 Anderson TM, McMahan JJ, Nwogu CE, Pombo MW, Urschel JD, Driscoll DL and Lele SB: Pulmonary resection in metastatic uterine and cervical malignancies. *Gynecol Oncol* 83: 472-476, 2001.
- 18 Seki M, Nakagawa K, Tsuchiya S, Matsubara T, Kinoshita I, Weng SY and Tsuchiya E: Surgical treatment of pulmonary metastases from uterine cervical cancer. Operation method by lung tumor size. *J Thorac Cardiovasc Surg* 104: 876-881, 1992.

P01-02

The expression of S100A14 into cytosol is associated with cervical cancer growth.

H.J. Kim¹, W.K. Yang¹, H.J. Kwon¹, E.S. Kang², J.H. Kim¹

¹Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (Korea, republic of), ²Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea (Korea, republic of)

Background / Objectives

Expression of S100A14 is associated with cancer growth, invasion, and migration is well known. However, it is not known that subcellular location of S100A14 is correlated with cervical cancer, yet. This study investigated the role of S100A14 along with subcellular location in cervical cancer.

Methods

To begin with, the subcellular location of S100A14 was confirmed by immunohistochemistry in cervical cancer tissue microarray. The mRNA and protein expression of S100A14 were measured via qPCR and western blotting, and subcellular location of S100A14 was confirmed via immunocytochemistry in cervical cancer cell lines. Then the growth of a cervical cancer based on the expression of S100A14 was measured through stably expressed cervical cancer cells including over-expression and sh-RNA knockdown of S100A14.

Results

The location of S100A14 according to progressing cancer was changed from membrane into cytosol in S100A14 stained cervical cancer tissue microarray. The subcellular location of S100A14 is into cytosol in cervical cancer HeLa and Caski cells, but is into membrane in immortal keratinocyte HaCaT cells. In the case of over-expressing S100A14, the cell growth is increased in HeLa and Caski cells. On the other hand, the cell growth is decreased in ME-180 cells while S100A14 expression was knock-down via over-expression of sh-S100A14.

Conclusion

The location of S100A14 expression was changed into cytosol from membrane while normal tissue in cervix progress to malignant cervical cancer. And, we verified that the control of S100A14 expression is associated with cancer growth in cervical cancer. These results suggest that S100A14 could be a feasible diagnostic marker via detecting location of expression and an effectual therapeutic target via controlled expression.

P01-03

PROGNOSTIC RELEVANCE OF MULTIPLE HPV GENOTYPES IN CERVICAL CARCINOMA

G. Lillsunde Larsson¹, M. Kaliff¹, G. Helenius¹, M.G. Karlsson¹, L. Bohr-Mordhorst², B. Sorbe²

¹Dep. of Laboratory medicine, Faculty of medicine and health, Örebro University (Sweden), ²Dep. of Oncology, Faculty of medicine and health, Örebro University (Sweden)

Background / Objectives

In cervical cancer, the high risk HPV genotypes according to IARC are well described as a cause for cancer. Less is known about the impact of multiple genotypes in cancer progression and also on accompanying low risk types. With refined methods that are now available, substantially more genotypes can be included and evaluated for prognostic relevance.

Methods

128 cervical cancer cases, treated with primary radiotherapy, were included in the study. Genotyping was performed on FFPE sample DNA with the Anyplex II HPV28 (Seegene) detecting 28 different genotypes, both high and low risk types.

Results

Results could be obtained from 121 of 128 samples. In total, 82% (99/121) were positive for HPV. HPV-16 was the most common genotype (n = 44) followed by HPV-18 (n= 11). Single infections were present in 82 cases and multiple infections in 17. Tumors with multiple infections were in general composed of two or more high risk types (n= 15), with 2 exceptions; HPV33+HPV42 and HPV56+ HPV53.

When comparing tumors with one genotype to tumors holding two or more genotypes; the overall recurrence-rate was 25.9% and 52.9% respectively (Pearson chi-square; P = 0.027). Distant recurrences were highly significantly (Pearson chi-square; P = 0.001) associated with presence of multiple HPV-genotypes 47.1% versus 12.9%. This group also had a worse cancer-specific survival rate (log-rank test; P = 0.023) compared to patients with tumors with one HPV genotype present.

Tumors containing HPV18 were associated with higher recurrence rate and more loco-regional recurrences compared to non-HPV 18 holding tumors (Pearson chi-square; P = 0.037 and 0.027).

However, no significant associations between prognosis and individual HPV-types or groups of HPV-types were found.

Conclusion

Cervical cancer treated with primary radiotherapy (external beam therapy and brachytherapy) and positive for multiple HPV-strains is associated with more recurrences as well as worse prognosis (cancer-specific survival rate) compared with cases containing single strain HPV.

P01-04

Application of Topical Imiquimod for Treatment Cervical Intraepithelial Neoplasia in Young Women: A preliminary result of a pilot study

J.H. Nam, P.S. Jung, D.Y. Kim

Asan Medical Center (Korea, republic of)

Background / Objectives

In young, especially nulliparous women, it is not easy to decide on excisional therapy for cervical intraepithelial neoplasia (CIN). We aimed to evaluate how effective topical imiquimod is in the treatment of high-grade CIN so that excisional therapy can be avoided in young women.

Methods

Patients with CIN were allocated to this pilot study. They did not want excisional therapy and agreed with topical imiquimod therapy, which required once-a-week hospital visit for 8 weeks for the application of imiquimod to the cervix by a gynecologic oncologist. If the lesion got worse during treatment, it was decided to convert imiquimod therapy to excisional therapy.

Results

A total of 36 patients with a median age of 29 years (range, 22–41 years) agreed to receive topical imiquimod therapy. Of these, 32 patients (88.9%) were positive for high-risk human papillomavirus (HR HPV). Twenty-five patients (69.4%) had low-grade squamous intraepithelial lesion (LSIL), and 11 (30.6%) had high-grade squamous intraepithelial lesion (HSIL) on their initial LBC. Twenty-eight patients underwent punch biopsy, which showed CIN 1 in 7 (19.4%), CIN 2 in 11 (30.6%), and CIN 3 in 10 (27.8%) patients. Twenty patients finished the 8-week imiquimod therapy. Among them, 14 patients had CIN 2 or 3, and 6 patients had CIN 1. HR HPV was positive in 12 patients. On the last examination, 14 patients (70.0%) had negative intraepithelial lesions, 3 (15.0%) had atypical squamous cells of undetermined significance, and 1 (5.0%) had LSIL. Two patients had persistent HSIL: 1 patient underwent loop electrosurgical excision procedure, resulting in CIN 3 with positive resection margin, and the other patient underwent punch biopsy, resulting in intermediate cells and restarted imiquimod therapy. Only 7 patients were negative for HR HPV.

Conclusion

This study showed that topical imiquimod therapy was effective for the treatment of high-grade CIN, with a histologic regression rate of 85.7% (14/20) and HPV eradication rate of 25.0% (8/32). Based on our findings, topical imiquimod therapy might have a successful therapeutic effect in young women with CIN 2-3 so that they can avoid excisional therapy. In addition, it could be a more reassuring treatment option for CIN 1 than just follow-up after few months. To confirm its efficacy, a phase II study with larger cohort would be needed.

P01-05

Thin HSIL of the Cervix: Detecting a Variant of High-Grade Squamous Intraepithelial Lesions with a p16INK4a - Antibody

O. Reich¹, S. Regauer²

¹Medical University of Graz, Dpt. Obstet/Gyn (Austria), ²Medical University of Graz, Institute for Pathology (Austria)

Background / Objectives

The WHO defines thin HSIL as a high-grade intraepithelial lesion of the cervix that is usually less than 10 cells thick. These lesions usually develop in early metaplastic squamous epithelium without antecedent LSIL. The prevalence of thin HSIL is not well documented. We evaluated different characteristics of thin HSIL at time of treatment.

Methods

We studied 25 formalin-fixed and paraffin-embedded conization specimens processed as step-serial sections. HSIL < 9 cells thick were classified as thin HSIL. HSIL >10 cells thick were classified as classic HSIL. Immunohistochemical p16ink4a staining was used to confirm lesions of thin HSIL.

Results

Overall, 19 (76%) specimens contained both thin HSIL and classic HSIL; 4 (16%) contained thin HSIL only; 1 (4%) contained classic type HSIL only; and 1 (4%) contained thin HSIL and LSIL. Thin HSILs developed in both the columnar surface epithelium and deep cervical glandular epithelium. Most thin HSILs were 5 cells thick. All HSILs (thin and classic) were located inside the transformation zone including the squamocolumnar junction and had a median horizontal extension of 8 (0.3-21) mm.

Conclusion

Our findings suggest that thin HSILs are frequent findings in cone specimens, that they coexist with classic HSIL, and preferably arise in the exposed parts of the transformation zone including the glandular crypts.

References

Stoler M, Bergeron C, Colgan TJ, et al. Tumours of the cervix: Squamous cell tumours and precursors. In: Kurman JR, Carcangiu ML, Herrington CS, et al. (eds). World Health Organization Classification of Tumours of the Female Reproductive Organs. 4th edition., IARC press, Lyon 2014, p172-182.

Reich O, Regauer S. Two major pathways of high-grade squamous intraepithelial lesions of the cervix. *Am. J. Surg. Path.* 2014; 38: 1579-1580.

Regauer S, Reich O. CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). *Histopathology* 2007; 50: 629-635.

P01-06

CERVICAL CANCER SURVIVORS AND HEALTH CARE USE: A POPULATION-BASED REGISTER STUDY

M. Skorstengaard¹, M.E. Frederiksen¹, M.V.P. Baillet¹, P.T. Jensen², C. Rygaard³, J. Hallas⁴, E. Lynge¹

¹Dept. of Public Health, University of Copenhagen (Denmark), ²Dept. of Gynaecology and Obstetrics, Odense Hospital (Denmark), ³Dept. of Pathology, Hvidovre Hospital (Denmark), ⁴Dept. of Clinical Pharmacology, Odense Hospital (Denmark)

Background / Objectives

Treatment of cervical cancer can have several complications such as urinary problems, abdominal pain, vaginal dryness, lymphedema and induced menopause, depending on the treatment. We tried to assess the use of health care in women who are diagnosed with and treated for cervical cancer.

Methods

We conducted a population-based register study including women with cervical cancer (exposed) diagnosed in Denmark in 2001-2005 compared to women without cervical cancer (non-exposed) in the same time period. As indicators for health care utilization we used the number of contacts to general practitioners (GP), hospitals and psychiatrists/psychologists and the dispensing of prescription drugs. The data were retrieved from several Danish registers. A five-year period “before” and a five-year period “after” the cervical cancer diagnosis were compared.

Results

In total, 926 women with cervical cancer and 1,004,759 women without cancer were included. Exposed women increased their number of contacts with 14.1 (95% CI: 10.3-17.8) and 4.18 (95% CI: 4.06-4.31) to the GPs and hospitals respectively from the “before” period to the “after” period. Non-exposed women increased their number of contacts over time to the GPs with 5.5 (95% CI: 5.3-5.6) and to the hospitals with 0.06 (95% CI: 0.056-0.064). Women with cervical cancer therefore have 8.6 and 4.12 more contacts to GPs and hospitals respectively than women without cervical cancer. An increase over time was seen for the use of prescription drugs with 385 defined daily doses (DDD) more for exposed women compared to non-exposed women. There was a slight increase in contacts to the psychologists/psychiatrists with limited difference of 0.50 more contacts for exposed women compared to non-exposed women.

Conclusion

Women with cervical cancer increase, as expected, their use of prescription drugs and the number of contacts to general practitioner and hospitals after the diagnosis compared to women without cancer. The small increase in number of contacts to psychologists/psychiatrists was unexpected. These results indicate that the diagnosis and treatment of cervical cancer, as handled in Denmark, do not lead to psychological side effects requiring attention from psychologists/psychiatrists.

P01-07

ESTIMATING THE MANAGEMENT COST OF CERVICAL INTRAEPITHELIAL NEOPLASIA IN THE UK

M. Uhart¹, **V. Coles**², **J. Jacob**³, **J. Gagnon**³, **F. Bianic**⁴, **A. Nordin**⁵, **N. Largeron**¹

¹Sanofi Pasteur MSD (France), ²Sanofi Pasteur MSD (United kingdom), ³Mapi (United kingdom), ⁴Mapi (France), ⁵Queen Elizabeth the Queen Mother Hospital (United kingdom)

Background / Objectives

There are 33,230 to 63,965 new cases of high-grade cervical precancerous lesions (cervical intraepithelial neoplasia, CIN2+) every year in the UK.[1] Even for lower grades, the psychological effect and economic impact of CIN diagnosis are substantial.[1] A nonavalent HPV vaccine is now licensed in Europe with the potential to prevent close to 90% of cervical cancers and approximately 50% of CIN1 and 80% of CIN2+.[1] Although there have been some published estimates of the costs associated with CIN, few data closely reflect current management guidelines in the UK.[2] With HPV triage recently recommended for cases of borderline and low-grade dyskaryosis, the management pathway continues to evolve and is increasingly branched, complicating the estimation of the economic burden. To address this limitation, this study aimed to understand the pathway and determine the costs associated with the management of HPV-related CIN1 and CIN2/3 in current UK practice.

Methods

A probabilistic decision tree was constructed according to current UK guidelines to simulate the management pathway of a woman diagnosed with CIN. An expert clinician validated the pathway to ensure that it reflected current clinical practice. The episode of care extended from the initial abnormal cytology up to either the progression of the lesion to cervical cancer or the resolution of the case and discharge of the woman to routine recall. All attributable treatment and follow up events were modelled, but long-term maternal consequences, such as preterm birth and neonatal morbidity were not considered. Unit costs associated with consultations, screening and diagnostic tests, and treatment were collected from national sources, as were epidemiological probabilities. Costs were also reported by pathway stages (i.e., screening, diagnosis, treatment, and follow-up) and by initial cytology (smear test) result.

Results

In the base case scenario, the average cost of care per episode was £759.47 for CIN 1 and £788.52 for CIN 2/3. Distribution of costs were similar across diagnosis, treatment, and follow-up stages for CIN 1 (£224.00, £209.96, £240.88), while treatment was responsible for 44% of costs in CIN 2/3 management. Stratified by initial smear test result, the average episode cost for a high grade smear (i.e. moderate to severe dyskaryosis) was £557.93, compared to £398.50 for a low grade smear (i.e. borderline to mild dyskaryosis).

Conclusion

Costs associated with an episode of care for CIN were reappraised according to current management algorithms, and found to be higher than a prior, regularly cited estimate.[3]

References

[1] Hartwig, S., et al., Estimation of the epidemiological burden of HPV-related anogenital cancers, precancerous lesions, and genital warts in women and men in Europe: Potential additional benefit of a nine-valent second generation HPV vaccine compared to first generation HPV vaccines. *Papillomavirus Research*, 2015. 1: p. 90-100.

[2] NHS Cancer Screening Programmes, NHS Cervical Screening Programme Screening Protocol Algorithm for HPV Triage and TOC 2014.

[3] Martin-Hirsch, P., et al., Management of women with abnormal cervical cytology: treatment patterns and associated costs in England and Wales. *BJOG.*, 2007. 114(4): p. 408-415.

P01-08

Dynamin 2 inhibitors as novel therapeutic agents in cervical cancer cells

L. Yoo-Young, K. Byoung-Gie, B. Duk-Soo, L. Jeong-Won, C. Chel Hun, K. Tae-Joong

Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea (Korea, republic of)

Background / Objectives

We investigate the possibility of dynamin 2 as a potential treatment target in cervical cancer cells using various dynamin 2 inhibitors.

Methods

Tissue microarray for the expression of dynamin 2 was performed in 208 early cervical cancer patients and analyzed the association between expression of dynamin 2 and primary tumor characteristics such as tumor size and depth of invasion. Then we performed in vitro using dynamin 2 inhibitors including MiTMAB, OcTMAB, Dynasore, and DD-6 on HeLa cells with proliferation, apoptosis, and migration assay.

Results

When we compare the expression level of Dynamin 2 based on the pathological findings, tumor size more than 2 cm and tumor invasion more than half of the entire cervix were associated with higher proportion of any dynamin 2 expression compared with no expression (+1, +2, and +3 vs. 0, $P = 0.013$, $P = 0.045$). All of dynamin 2 inhibitors including MiTMAB, OcTMAB, Dynasore, and DD-6 significantly decreased proliferation and increased apoptotic activity in HeLa cells. And also these all inhibitors significantly decreased MMP-9 expression compared with control in HeLa cells. In migration assay, dynasore and DD-6 decreased migration in HeLa cells coated by laminin 1. However, DD-6 most strongly decreased migration performance in fibronectin-coated wells.

Conclusion

Targeting dynamin 2 with various specific inhibitors may be a promising new approach for the treatment of cervical cancer in the future.

References

1. Hirte H, Kennedy EB, Elit L, Fung Kee Fung M. Systemic therapy for recurrent, persistent, or metastatic cervical cancer: a clinical practice guideline. *Current oncology (Toronto, Ont)*. 2015 Jun;22(3):211-9. PubMed PMID: 26089720. Pubmed Central PMCID: PMC4462531. Epub 2015/06/20. eng.
2. Lee YY, Song SY, Do IG, Kim TJ, Kim BG, Lee JW, et al. Dynamin 2 expression as a biomarker in grading of cervical intraepithelial neoplasia. *European journal of obstetrics, gynecology, and reproductive biology*. 2012 Oct;164(2):180-4. PubMed PMID: 22959143. Epub 2012/09/11. eng.
3. Lee YY, Do IG, Park YA, Choi JJ, Song SY, Kim CJ, et al. Low dynamin 2 expression is associated with tumor invasion and metastasis in invasive squamous cell carcinoma of cervix. *Cancer biology & therapy*. 2010 Aug 15;10(4):329-35. PubMed PMID: 20574164. Epub 2010/06/25. eng.
4. Lee YY, Kim TJ, Kim JY, Choi CH, Do IG, Song SY, et al. Genetic profiling to predict recurrence of early cervical cancer. *Gynecologic oncology*. 2013 Dec;131(3):650-4. PubMed PMID: 24145113. Epub 2013/10/23. eng.
5. Baldassarre M, Pompeo A, Beznoussenko G, Castaldi C, Cortellino S, McNiven MA, et al. Dynamin participates in focal extracellular matrix degradation by invasive cells. *Molecular biology of the cell*. 2003 Mar;14(3):1074-84. PubMed PMID: 12631724. Pubmed Central PMCID: PMC151580. Epub 2003/03/13. eng.
6. Chao WT, Kunz J. Focal adhesion disassembly requires clathrin-dependent endocytosis of integrins. *FEBS letters*. 2009 Apr 17;583(8):1337-43. PubMed PMID: 19306879. Pubmed Central PMCID: PMC2801759. Epub 2009/03/25. eng.
7. Bhattacharya R, Kang-Decker N, Hughes DA, Mukherjee P, Shah V, McNiven MA, et al. Regulatory role of dynamin-2 in VEGFR-2/KDR-mediated endothelial signaling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2005 Oct;19(12):1692-4. PubMed PMID: 16049137. Epub 2005/07/29. eng.
8. Lee S, Jung KY, Park J, Cho JH, Kim YC, Chang S. Synthesis of potent chemical inhibitors of dynamin GTPase. *Bioorganic & medicinal chemistry letters*. 2010 Aug 15;20(16):4858-64. PubMed PMID: 20621477. Epub 2010/07/14. eng.
9. Stowell MH, Marks B, Wigge P, McMahon HT. Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring. *Nature cell biology*. 1999 May;1(1):27-32. PubMed PMID: 10559860. Epub 1999/11/13. eng.

10. Praefcke GJ, McMahon HT. The dynamin superfamily: universal membrane tubulation and fission molecules? *Nature reviews Molecular cell biology*. 2004 Feb;5(2):133-47. PubMed PMID: 15040446. Epub 2004/03/26. eng.
11. Schelhaas M, Shah B, Holzer M, Blattmann P, Kuhling L, Day PM, et al. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. *PLoS pathogens*. 2012;8(4):e1002657. PubMed PMID: 22536154. Pubmed Central PMCID: PMC3334892. Epub 2012/04/27. eng.
12. Smith JL, Campos SK, Ozbun MA. Human papillomavirus type 31 uses a caveolin 1- and dynamin 2-mediated entry pathway for infection of human keratinocytes. *Journal of virology*. 2007 Sep;81(18):9922-31. PubMed PMID: 17626097. Pubmed Central PMCID: PMC2045393. Epub 2007/07/13. eng.
13. Xu B, Teng LH, Silva SD, Bijian K, Al Bashir S, Jie S, et al. The significance of dynamin 2 expression for prostate cancer progression, prognostication, and therapeutic targeting. *Cancer medicine*. 2014 Feb;3(1):14-24. PubMed PMID: 24402972. Pubmed Central PMCID: PMC3930386. Epub 2014/01/10. eng.

P01-09

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR - C AND PODOPLANIN IN SQUAMOUS INTRAEPITHELIAL LESION

P. Belfort Mattos¹, G. Focchi², J. Ribalta³, N. Gois Speck³

¹PhD Universidade Federal de São Paulo - Gynecology Department (Brazil),

²Doctor Universidade Federal de São Paulo - Pathology Department (Brazil),

³Doctor Universidade Federal de São Paulo - Gynecology Department (Brazil)

Background / Objectives

Vascular endothelial growth factor - C (VEGF-C) and podoplanin has been identified as lymphangiogenesis regulators and might be essential to restrict tumor growth, progression, and metastasis.^{1,2,3,4,5,6,7,8,1,2,3,4,5,6,7,8} The aim of the study was to evaluate the molecular and clinicopathological profiles of VEGF-C and podoplanin expression in neoplastic cells of intraepithelial uterine cervical lesion.

Methods

A total of 234 paraffin-embedded normal and abnormal cervical tissue (CIN) samples were studied. Immunohistochemical expression of VEGF-C and podoplanin in histologic sections of tissue microarray were performed, which were used the monoclonal antibodies anti-VEGF C1 and anti-Podoplanin clone D2-40. Co-expression among the antibodies was assessed and the profiles of immunodetection were associated with clinicopathological data.

Results

The positive staining rates of VEGF-C in 191 cervical neoplasia specimens were 18.9% to CIN 1, 25% to CIN 2 and 48.7% to CIN 3, and in 43 normal tissues (4.7%). These expressions were significantly associated with clinicopathologic parameters ($p < 0.001$). Comparing the groups and intensity of immunostaining of podoplanin observe statistical significance, the negative focal expression was more present in the CIN 3 compared to CIN 1, CIN 2 and control ($p = 0.016$). The correlation of the immunoreactivity of the two factors, podoplanin was also common in many types of VEGF-C in the control group, CIN 1 and CIN 2. Moreover, patients with CIN 3 had strong staining for VEGF-C and low staining for podoplanin ($p = 0.018$).

Conclusion

The findings indicated that VEGF-C expression seems to be different in degrees of CIN. Podoplanin expression was lower in CIN 3. VEGF-C high expression may be a tendency in patients with high-grade lesions when compared with podoplanin. The results herein presented provide additional evidence of the simultaneous examination of VEGF-C and podoplanin immunoeexpressions that it will benefit the diagnosis, prevention of cancer cells growth and spread and determine the treatment strategy in patients with CIN.

References

1. Al-Rawi MAA, Jiang WG. Lymphangiogenesis and cancer metastasis. *Front Biosci.* 2011 Jan;16:723-39.
2. Chaudary N, Milosevic M, Hill RP. Suppression of vascular endothelial growth factor receptor 3 (VEGFR3) and vascular endothelial growth factor C (VEGFC) inhibits hypoxia-induced lymph node metastases in cervix cancer. *Gynecol Oncol.* 2011 Nov;123(2):393-400.
3. Chung JC-Y, Shum-Tim D. Neovascularization in tissue engineering. *Cells.* 2012 Dec;1:1246-1260.
4. Cimpean AM, Raica M. Lymphangiogenesis and Inflammation-Looking for the "Missing Pieces" of the Puzzle. *Arch Immunol Ther Exp (Warsz).* 2015 Jul [Epub ahead of print].
5. Doobar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. *Rev Med Virol.* 2015;25:2-23.
6. Liu H, Xiao J, Yang Y, Liu Y, Ma R, Li Y, et al. COX-2 expression is correlated with VEGF-C, lymphangiogenesis and lymph node metastasis in human cervical cancer. *Microvasc Res.* 2011 Sep;82(2):131-40.
7. Longatto-Filho A, Pinheiro C, Pereira SMM, Etlinger D, Moreira MAR, Jubé LF, et al. Lymphatic vessel density and epithelial D2-40 immunoreactivity in pre-invasive and invasive lesions of the uterine cervix. *Gynecol Oncol.* 2007 Oct;107(1):45-51.
8. Maturana-Ramírez A, Espinoza I, Reyes M, Aitken JP, Aguayo F, Hartel S, et al. Higher blood density in comparison to the lymphatic vessels in oral squamous cell carcinoma. *Int J Clin Exp pathol.* 2015;8(10):13677-13686.

P02-01

COUNTERFACTUALS OF DISEASE DISCOVERY WITHIN THE FRIDA STUDY

E. Barrios¹, E. Franco², M. El-Zein², E. Lazcano-Ponce³, L. Torres-Ibarra¹, R. Hernández¹, B. Paredes¹, L. León⁴, J. Salmerón⁵

¹Escuela de Salud Pública de México, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México (Mexico), ²Division of Cancer Epidemiology, McGill University, Quebec, Canada (Canada), ³Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México (Mexico), ⁴CONACyT Research Fellow, Instituto Nacional de Cancerología, Ciudad de México, México (Mexico), ⁵Unidad de Investigación Epidemiológica y en Servicios de Salud, Instituto Mexicano del Seguro Social, Cuernavaca, Morelos, México (Mexico)

Background / Objectives

Colposcopy tends to be subjective given the weak correlations between visual changes and disease severity. It has been suggested that systematic collection of cervical biopsies (SCCB) may increase the detection of high-grade cervical intraepithelial neoplasia and/or cancer compared to the traditional directed cervical biopsies (DCB) approach. We aim to describe through stochastic simulation of cervical dysplasia the methodology and statistical strategy for evaluating the gain in disease detection consequent to the use of SCCB compared to DCB based on data from the FRIDA study.

Methods

The FRIDA study, conducted in the Tlaxcala State Ministry of Health, Mexico, was designed to evaluate at the population level new triage alternatives for colposcopy evaluation among high-risk HPV (hrHPV) positive women aged 30 to 64 years. The current analysis uses data from the first 30,000 women enrolled. All participants were screened by hrHPV testing with a prevalence of 11%. Additional triage tests (hrHPV 16/18, cytology, OncoE6 protein, etc.) were done on hrHPV positive women. After the colposcopy examination in each quadrant, if Reid Index was ≤ 2 points a biopsy was not required, but necessary if evaluation was >2 points. One biopsy was collected from the most abnormal zone of the squamocolumnar junction; all biopsy collection sites were recorded. This procedure is referred as a SCCB. We performed stochastic models for counterfactual analyses, simulating a comparison between what actually happened and what would have happened in the absence of SCCB. We generated 3 different guideline-based scenarios reconstructing the main results observed in the FRIDA study, starting with the simplest scenario (hrHPV testing and cytology) and adding several levels of complexity (HPV 16/18 genotyping, cytology, DCB and SCCB based-colposcopy) while maintaining preceding scenarios. Stochastic analyses of scenarios 2 (randomization

according to HPV genotyping and cytology with DCB) and 3 (randomization according to HPV genotyping and cytology with SCCB) will enable the calculation of detection rates with respect to different collection procedures of cervical biopsies. Histology results were used to reflect the detection rate and to determine the incremental gain in the diagnosis of cervical disease.

Conclusion

This analysis will investigate the benefits of incorporating the SCCB approach meanwhile will provide valuable information to policy makers that will contribute to the introduction of this strategy as a standard practice in colposcopy evaluation in Mexico; this must be guided by evidence obtained from the local context about the cost-effectiveness of the intervention.

References

1. Torres-Ibarra L y col. Triage strategies in cervical cancer detection in Mexico: methods of the FRIDA Study. *Salud Pública de México* / vol. 58, no. 2, febrero de 2016.

P02-02

SENSITIVITY AND SPECIFICITY OF CYTOLOGY AND COLPOSCOPY COMPARED TO FINAL HISTOLOGY AFTER LLETZ

D. Tsolakidis, A. Vatopoulou, S. Pitis, E. Klonos, A. Papanikolaou

1st Dept Obstetrics and gynecology Aristotles University of Thessaloniki (Greece)

Background / Objectives

Large loop excision of the transformation zone (LLETZ) is an established method for the treatment of CIN since it combines the advantages of an outpatients procedure with excision of the lesion and histologic confirmation.

Objective is to compare the sensitivity and specificity of abnormal cytology \geq ASCUS and colposcopy vs the histologic results of punch biopsy and loop cone specimen .Information is presented on excision margins and recurrence of the lesion.

Methods

Retrospective study of 129 patients , aged $33 \pm 9,3$ yrs over a 4 yrs period ,that underwent LLETZ for cervical dysplasia. The specificity and sensitivity of cervical smears \geq ASCUS and colposcopy were compared for the diagnosis of low grade or high grade lesions as diagnosed by final histology. Statistical significance was calculated with t test.

Results

Of 129 patients 98 underwent punch biopsy and more and 63 had an excisional procedure . The sensitivity and specificity of cervical smear for LGSIL was 40% and 75% and of colposcopy was 90% and 76,7%. The sensitivity and specificity of cervical smear for HGSIL was 40,7% and 80% and of colposcopy was 78% and 80% .Colposcopy was more accurate than cytology in the correct diagnosis of SIL ($p=0.0002$) . The final histology was LGSIL in 12 patients (19%) ,HGSIL in 41 (65%) ,invasive cancer in 3 (5%) and no lesion in 8 (13%). Two patients had positive margins (3%) and 2 (3%) recurred after a median follow up of 20 months.

Conclusion

Colposcopy is a more accurate method for assessing SIL. Histologic confirmation by an excisional procedure is an effective method of treatment of SIL.

P03-01

FISHER CONE BIOPSY IN THE MANAGEMENT OF CERVICAL CANCER PRECURSORS

A. Dörr¹, M. Kozák¹, E. Sehnálková², A. Šustek¹, P. Vytríšalová¹, M. Fabian¹, P. Lucan¹, M. Martinková¹, A. Šefčíková¹, L. Šimková¹, P. Hyklová¹, V. Smetansky¹, J. Malaník¹

¹Department of Obstetrics and Gynecology, Silesian Hospital Opava, Olomoucká 86, 746 01 Opava (Czech republic), ²Department of Pathology, Silesian Hospital Opava, Olomoucká 86, 746 01 Opava (Czech republic)

Background / Objectives

The goal of this study is to analyse the accuracy of cytologic and colposcopic findings and compare it with the results of histology by LEEP or Fisher conisation.

Methods

Material of clinical studies of 230 patients with cervical pathology has been analysed.

They were assigned to treatment by Fisher cone biopsy excisor or other excisional methods of the cervix.

Eligibility criteria included CIN 2 or 3 detected by punch biopsy or discrepancies between cytology, biopsy and colposcopy examination or long time persistent CIN1.

A pathologist analysed the degree of neoplasia, specimens for margin interpretability and adequacy of excision as well as HPV testing.

Results

LEEP method have some disadvantages, included electrocautery artifacts or fragment pieces. That led to difficulties with safety evaluations of gained margins.

Specimens with interpretable margins were in 94% of all cases. In 6% of cases we found out recurrence of CIN and in 4% cases CIS of the cervix, in some cases in the endocervical location.

No problems in course of next pregnancy in the cases of Fisher cone biopsy were observed

Conclusion

Method of Fisher conisation appears to be a safe and reliable method for diagnosis, treatment and thrifty towards next pregnancy possibility.

P03-02

OBSTETRIC OUTCOME AFTER CERVICAL CONIZATION OR LLETZ: A RETROSPECTIVE STUDY IN THE UNIVERSITY HOSPITAL OF LEUVEN

H. Verschuere¹, W. Poppe¹, M. Arbyn²

¹Unit Gynaecology - Obstetrics, University Hospital Leuven, Leuven (Belgium),

²Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels (Belgium)

Background / Objectives

The purpose of this study was to investigate the association between the treatment for CIN with cervical conization or LLETZ and the obstetric outcome. The main obstetric outcome was gestational age of delivery, neonatal biometry and neonatal condition at birth.

Methods

Data was collected from 1382 women who received a conization between 2004 and 2012. In this group 108 patients were identified whom had a subsequent delivery. 22 women had two subsequent deliveries, 2 women had 3 subsequent deliveries. A control group was composed based on a match for every patient by maternal age at delivery, year of delivery and parity.

Results

Only a significant lower birth weight could be found (3118 ± 607 vs. 3299 ± 646 $P < 0.01$) in the study group. Non-significant higher rates of preterm delivery (RR 1.42 CI 0.75 – 2.71) and severe preterm delivery (RR 3 CI 0.83 – 10.84) were found in the treatment group. There was no significant difference in gestational age of delivery (270 ± 19 vs. 272 ± 17 $P = 0.14$), neonatal length (49 ± 3 vs. 50 ± 3 $P = 0.09$) and neonatal head circumference (34 ± 2 vs. 34 ± 2 $P = 0.48$) between the study group and the control group.

Conclusion

Treatment of CIN by performing conization of LLETZ did not significantly affect the gestational age of delivery in the University Hospital of Leuven. However a significant lower birth weight could be found in the study group. Although matching, residual confounding cannot be excluded.

P04-01

CANCER SCREENING OF CERVICAL PREGNANCY IN MATERNITY CENTER HEALTH NABIL CHOUCAIR AND HOSPITAL INSTITUTE SOCIAL IN DAKAR (SENEGAL) : ABOUT 67 CASES

O. Gassama, M.M. Niang, M.E. Faye Dieme, P.M. Moreira, A. Diouf, J.C. Moreau

Gynecology and Obstetrics, Teaching Hospital Aristide Le dantec, Dakar, Senegal (Senegal)

Background / Objectives

Develop the epidemiological profile of patients who received Pap smears during pregnancy;

Describe aspects of cytological smears performed in pregnant women;

Describe the therapeutic management in case of anomalies in the cervical smear during pregnancy.

Methods

This was a prospective, descriptive and analytical conducted from 15.01.2015 to 06.31.2015 at maternity of Nabil Choucair Health Center and the Institute Hospital institute social in Dakar. The Pap smear was performed in all patients after having given their consent explanation.

Results

The epidemiological profile of our patient was a paucipare gestity with an average of 3 with extremes ranging from 1 to 7, an average parity of 2.4 with extremes ranging from 1 to 7. In our series all patients were married and had their first sexual intercourse at 21.7 years. The average time of pregnancy was 15.4 SA with extremes ranging from 6-32 SA. In history, only 5 patients (7.4%) had achieved a Pap smear. The Pap smear during pregnancy was normal in 88.7% and found abnormalities in 11.3%. The anomalies found mainly interested squamous cells and were divided into low-grade lesions in 57.1% and abnormal squamous cells of undetermined significance in 42.1%. Colposcopy was normal and satisfactory in 4 patients (50%) and found a unique transformation of degree 1 in 2 patients (25%) and a unique transformation of degree 2 in 2 patients (25%). Therapeutically a loop diathermy conization the associated banding was performed for severe

dysplasia or injury the pathologist could not eliminate micro-invasion. In the postpartum period, all dysplastic cervical lesions diagnosed during pregnancy had regressed.

Conclusion

Pregnancy is an extraordinary opportunity to screen for cervical cancer during antenatal care

P04-02

Clinical Significance of Atypical Glandular Cells on Cytology

H.J. Kim¹, W.K. Yang¹, H.J. Kwon¹, E.S. Kang², J.H. Kim²

¹Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (Korea, republic of), ²Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea (Korea, republic of)

Background / Objectives

To evaluate the histologic outcomes and clinical significance of patients with atypical glandular cells detected either on conventional smears and liquid based cytology.

Methods

A retrospective analysis of the pathology database of AGC diagnosed on cytology between 2000 and 2016 in seven tertiary medical centers in Korea. Cytohistological correlation of 131 patients was performed. Significant lesions included high-grade squamous intraepithelial lesion, adenocarcinoma in situ (AIS), cervical cancer, endometrial hyperplasia (EH), endometrial cancer and other malignancies.

Results

Mean age was 46.4 years. 60 (45.8%) of 131 patients were confirmed as having clinically significant lesions comprising cervical intraepithelial neoplasia (CIN) II-III (9.2%), squamous cell carcinoma in situ (CIS) (4.6%), adenocarcinoma in situ (AIS) (8.4%), cervical cancer (15.3%), endometrial hyperplasia (0.8%), endometrial cancer (6.1%) or other malignancies (1.5%). The prevalence of significant pathologies in women with AGC was significantly higher compared with normal cytologic diagnosis.

Conclusion

This study highlights the clinical importance of the AGC on cytology associated with underlying significant pre-invasive and invasive lesions. So more aggressive assessment strategy for AGC is warranted.

P04-03

Management of abnormal pap smear during pregnancy

A.C. Porn, L. Brandi, T. Fehm, M. Hampl

University Hospital Duesseldorf (Germany)

Background / Objectives

The management of patients with abnormal pap smear in pregnancy is a challenge for gynaecologists. It is therefore recommended to send these women to a Dysplasia Clinic for further diagnostic procedures. 1- 7% of all pregnant women are diagnosed with an abnormal pap smear during the first visit of pregnancy. The aim is to guarantee the health of the mother on the one hand side and the prolongation of the pregnancy on the other hand side. Aim of this study was to investigate the outcome of pregnant women diagnosed and treated for abnormal pap smear at the dysplasia clinic of the University Hospital in Duesseldorf, Germany.

Methods

In this study we evaluated retrospectively the data of 102 patients visiting our dysplasia clinic from 2010 to 2015 in respect to cytology (Münchener NomenklaturII/III and Bethesda), HPV-infection status, colposcopy, histology, way of delivery and findings after delivery.

Results

The median age was 30.6 years; the mean age of gestation at first visit was 17.7 weeks. 39.2 % presented with PAPIIID (LSIL), 53.9 % with PAP IVa (HSIL), 1.9 % with PAP IIp (ASC-US), 2.9 % with PAP IIIp/g (ASC-H, AGC) and 0.98% with PAP IV b (HSIL with features suspicious for invasion). In 82 patients we diagnosed a major change lesion on colposcopy; in 46 cases we performed a biopsy to exclude invasion. In 39 cases histology confirmed a CIN3, in 6 cases the histological diagnosis was CIN1 or 2, one patient was diagnosed with microinvasive cervical cancer. 24.5 % were delivered via caesarean section (for other reasons than dysplasia), 75.5 % had a vaginal delivery. After delivery 58.2% received a LEEP because of persistence of CIN 3. During follow up visit after delivery 46.1 % showed regression, 51 % persistence of the dysplasia and 2.9 % had progressive disease. None showed a progression to invasive carcinoma.

Conclusion

These results support the recommendation of the guidelines of conservative management of abnormal pap smear in pregnancy. From our point of view a more invasive investigation such as a conisation is generally not recommended.

P04-04

HUMAN PAPILLOMAVIRUS (HPV) PREVALENCE AND CORRELATION WITH ABNORMAL CERVICAL CYTOLOGY FINDINGS IN WOMEN FROM CROATIA: A COMPOUNDING EFFECT OF LOW-RISK/HIGH-RISK HPV CO-INFECTION

Z. Profozic, T. Mestrovic, D. Kovacevic, V. Profozic

Polyclinic „Dr. Zora Profozic”, Bosutska 19, 10 000 Zagreb (Croatia)

Background / Objectives

Our aim was to determine the prevalence and age distribution of low-risk (LR) and high-risk (HR) HPV infection (as well as co-infection with both types) in female population primarily from the Zagreb region (Croatia), as well as to evaluate association of HPV positivity with abnormal cervical cytological findings.

Methods

The study involved a total of 422 women (aged 18-67) who approached our outpatient clinic in Zagreb during a 5-year period. Cervical scrapings for the detection of HPV DNA and for cytological evaluation were collected. Digene HC2 HPV DNA test (Qiagen Corporation, USA) was employed in screening specimens for LR and HR HPV types. The cytology was reported using Bethesda system and in accordance to Uniform Classification of Uterine Cervix Cytological Findings in Croatia "Zagreb 2002".

Results

Total HPV prevalence in our study population was 48.10%. Among HPV-positive women, 18.22% were positive for only LR HPV, 62.07% were positive for only HR HPV, whereas 19.70% were positive for both LR and HR HPV. From 2009 to 2013 a continuous rise in HPV prevalence was observed (42.75% in 2009 to 57.14% in 2013); similar (but less linear) trend was seen for HR HPV infection and co-infection with LR and HR HPV, while LR HPV infection has shown pronounced yearly variations. HPV positivity was significantly more prevalent in younger female examinees (18-30 age group) when compared to HPV-negative women ($p=0.0122$).

Correlation with cervical cytology revealed no statistically significant differences in the frequency of inflammatory changes ($p=0.7587$), parakeratosis/hyperkeratosis ($p=0.5959$) and ASC-US ($p=0.0997$) between HPV-positive and HPV-negative women. Cytologic changes associated with HPV, CIN I (or LSIL) and CIN II/III (or HSIL) were more frequently observed in HPV positive than in HPV negative women ($p<0.0001$, $p<0.0001$ and $p=0.0001$, respectively).

Statistically significant differences within HPV positive groups were found for the category of cytological changes associated with HPV ($p=0.0021$) and CIN I ($p=0.0184$); more specifically, both of these cytological classifications were more commonly observed in women co-infected with both LR and HR HPV types than in those solely infected with either LR or HR HPV.

Conclusion

Co-infection with both LR and HR HPV can have a compounding effect in the occurrence of changes associated with HPV and CIN I in Pap smears, as our results have shown that those abnormal cytological findings were more than twice as common in co-infection than in cases of infection with only LR or HR HPV. Hence LR HPV can act as a significant co-factor in the development cytological abnormalities.

P04-05

Cytological evaluation in penile samples with human papillomavirus

K. Shigehara¹, K. Nakashima¹, H. Iwamoto¹, H. Yaegashi¹, S. Kawaguchi¹, T. Nakashima², M. Shimamura³, M. Namiki¹

¹Kanazawa University Graduate School of Medical Science (Japan), ²Ishikawa Prefectural Central Hospital (Japan), ³Nomi City Hospital (Japan)

Background / Objectives

Although male HPV infection is frequent prevalent similar to women, less information regarding cytological findings in male samples and pathogenesis of HPV infection for men have been available. The present study was performed to determine the associations between human papillomavirus (HPV) infection and cytological changes in the penile samples.

Methods

The rubbed samples of the glans were collected from 142 patients with urethritis, and the collected cells were placed into preservative solution for liquid-based cytology. DNA was extracted from all samples, and β -globin gene amplification, HPV-DNA test, and HPV genotyping were performed. Among 46 HPV-positive samples, a papanicolaou staining was performed to evaluate cytological findings. Cytological findings were assessed based on 9 non-classic signs, which are known to suggest HPV infection.

Results

High-risk HPV and low-risk HPV were detected in 36 and 12 cases, respectively. Cytological signs of HPV infection were observed in 52% of high-risk HPV-positive samples, which is significantly higher compared to that in low-risk HPV-positive samples. As the HPV-infection associated cytological abnormal findings, perinuclear halo, hyperkeratocytosis, mild koilocytosis, and hyperchormatism were most frequently observed. Cytological atypia suspected to indicate penile intraepithelial neoplasia (PIN) were observed in 12 cases (PIN1, 10 cases; PIN2, one case). In situ hybridization demonstrated the presence of HPV-DNA in the morphologically abnormal cells in 31% of high-risk HPV-positive samples. High-risk HPV and low-risk HPV were detected in 36 and 12 cases, respectively. Cytological signs of HPV infection were observed in 52% of high-risk HPV-positive samples, which is significantly higher compared to that in low-risk HPV-positive samples. As the HPV-infection associated cytological abnormal findings, perinuclear halo, hyperkeratocytosis, mild koilocytosis, and

hyperchromatism were most frequently observed. Cytological atypia suspected to indicate penile intraepithelial neoplasia (PIN) was observed in 12 cases (PIN1, 10 cases; PIN2, one case). *In situ* hybridization demonstrated the presence of HPV-DNA in the morphologically abnormal cells in 31% of high-risk HPV-positive samples.

Conclusion

Cytological changes similar to cervical intraepithelial neoplasia in females could be detected in the HPV-positive penile samples.

References

1. Shigehara K, *et al.* Prevalence of human papillomavirus infection in the urinary tract of men with urethritis. *Int J Urol* 2010;**17**: 563-8.
2. Shigehara K, *et al.* Etiologic role of human papillomavirus infection in bladder carcinoma. *Cancer* 2011; **117**: 2067-76.
3. Kawaguchi S, Shigehara K, *et al.* Liquid-based urine cytology as a tool for detection of human papillomavirus, *Mycoplasma* spp., and *Ureaplasma* spp. in men. *J Clin Microbiol* 2012; **50**: 401-6.

P04-06

RESULTS OF CONVENTIONAL CYTOLOGY PAP SMEAR VERSUS LIQUID-BASED CYTOLOGY ASSOCIATED WITH HPV DNA TESTING IN WOMEN WITH UTERINE CERVICAL ECTOPY.

S.M. Fernandes, N. Speck, J. Ribalta

Federal University of São Paulo (Brazil)

Background / Objectives

Conventional cytology Pap smear; Co-testing; Ectopy. To analyze the cytological findings in conventional cytology Pap smear (CPS) and Liquid-based cytology (LBC) with HPV testing (Co-testing) in women with ectopy in reproductive age.

Methods

From November 2010 to June 2015, we selected 53 women in reproductive age, with ectopy confirmed by colposcopy, without therapeutic intervention, negative CPS for malignancy and no signs suggestive of cytopathic effect by the Human Papilloma Virus (HPV). Women treated with abrasive or surgical techniques, pregnant, lactating and immunosuppressed in general were not included. We collect new samples for LBC and HPV DNA test (Cobas®, Roche), in Thin Prep® jars and processed by private laboratory. Both cytological reports obeyed Naming System Bethesda 2001. We confronted the reports, the representation of the epithelia, vaginal microbiota and the positivity of HPV testing correlated with risk factors. We offered free informed consent and the study was approved by the Research Ethics Committee.

Results

The mean values in years for age, menarche, first sexual intercourse, interval between menarche and first sexual intercourse were 25.6; 12.2; 17.8 and 4.6 respectively. For parity 0.6 children and 3 for number of partners; 79% used hormonal contraceptives and 98% were non-smokers. The symptoms occurred in 50.9%, of which discharge 44.4%, pelvic pain 11%, postcoital bleeding 7.4% and their association 37%. The result of the CPS was 96.2% reactive/reparative and 3.8% Normal; 58.5% of squamous and glandular epithelia, 22.6% of the 3 epithelia, 17% squamous and columnar epithelium 1.9%. LBC revealed 83% reactive / reparative, 15% ASC-US and 2% LIEBG; of which 79.3% of the

samples contained representation of the 3 epithelia and squamous only 20.7%. The HPV DNA test was positive in 34%, 33.3% of women aged 25 or more. The average age of the group HPV positive was statistically less than the HPV negative ($p = 0.017$). The microbiota in the CPS was 47% *Lactobacillus sp, bacilli* and other *bacilli* 28.3% and bacterial vaginosis (BV) 1.9% and LBC was *Lactobacillus sp* 24.5%, *bacilli* 39.6%, mixed 17%, and BV 11.3%.

Conclusion

The study shows the incidence of ectopy in young women, nulliparous, symptomatic predominantly of vaginal discharge, hormonal contraceptive users, but non-smokers. The negative and inflammatory report was the most frequent in both cytological methods, however LBC showed no change detected in CPS by 17%. The isolated finding of the squamous epithelium in women with ectopy was higher at LBC. The deviation of the microbiota to BV was higher in LBC. The positivity for high risk HPV DNA was significant in patients younger than 25 years.

References

1. Machado Junior LC, Dalmaso AS, Carvalho HB. Evidence for benefits from treating cervical ectopy: Literature review. Sao Paulo Med J 2008;126:132–139.
2. Monroy OL, et al Prevalence of human papillomavirus genotypes, and mucosal IgA anti-viral responses in women with cervical ectopy. Journal of Clinical Virology 47 (2010) 43–48.
3. Junior JE, Giraldo PC, Gonçalves AK, do Amaral RL, Linhares IM. Uterine cervical ectopy during reproductive age: cytological and microbiological findings. Diagn Cytopathol. 2014 May; 42(5):401-4.
4. Solomon D, Davey D, Kurman R, et al.; forum group member: Bethesda 2001 workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002; 287:2114–2119.
5. Singh VB, Gupta N, Nijhawan R, Srinivasan R, Suri V, Rajwanshi A. Liquid- based cytology versus conventional cytology for evaluation of cervical Pap smears: Experience from the first 1000 split samples. J Pathol Microbiol 2015; 58: 17-21.
6. Buntinx F, Essed GG, Van der Putten HW, Buchholz RF, Arends JW, Knottnerus JA. Composition of cervical smears in patients with and without a cervical ectropion. J Clin Pathol 1995; 48:408–409.
7. Saslow et al/ Screening Guidelines for the Prevention and Early Detection of Cervical Cancer Am J ClinPathol012;137:516-542.

P04-07

A population-based study on cervical cancer risk by screening history

H.C. Chen, J.J. Lin, H.C. Hsu, C.A. Chen, J.Y. Tzeng

National Taiwan University (Taiwan, republic of china)

Background / Objectives

Cervical cancer is the fourth most common cancer in women, and 528,000 new cases were diagnosed worldwide in 2012. Pap smear is the major screening tool, but the screening interval varied by countries. Free-of-charge Pap has been provided to women aged 30 or over in Taiwan since 1995 annually, and recommendation was made for women to receive a Pap at least once within three years. The screening intervals should be evaluated according the evidence.

Methods

Women aged 30 or over and alive in Taiwan, in 2009 were enrolled. A total of 7,411,454 female residence without cervical cancer history were eligible. In 2010-2012, 5,141 invasive cervical cancer were diagnosed. The 3-year cervical cancer risk was calculated by stratification of women's Pap history from 1995 through 2009. According women received Pap or not in 2007, 2008 and 2009, they were classified into 8 groups. Among women received only one Pap in 2009, women were further defined into several sub-groups according the interval between their last Pap, such as 2-year (last Pap in 2007), 3-year (last Pap in 2006), et al. We aim to examine the cervical cancer risks across those groups with various screening intervals of 2-year, 3-year, 4-year or longer.

Results

In 2007-2009, 3,731,557 women received 5,969,422 Paps. In the period, 567,635 women received annual Pap, their 3-year cervical cancer risk was 25.4. The risk for those women participated in screening in any two years was ranged from 27.2 to 32.0. Regarding women received only one Pap in 2007, 2008, 2009, the risk was 46.3, 34.4 and 42.3. The highest risk was 95.6, among those did not received any Pap in 2007-2009. The risk for the interval of last Pap in 2-, 3-, 4-, 5- and 6-year was 27.2, 32.0, 37.5, 49.5 and 61.5, respectively. Compare to the annual Pap group, the corresponding odds ratio was 1.13 (95%CI=0.88-1.45), 1.26 (95%CI=0.95-1.68), 1.47 (95%CI=1.06-2.05), 1.96 (95%CI=1.38-2.80) and 2.44 (95%CI=1.6-3.68), respectively.

Conclusion

Based on the evidence from our population study, cervical cancer risk increased while the intervals of two Pap increased. No significant risk was observed among annual screening, 2-year and 3-year of intervals. But a substantial increase of risk was detected when the interval was longer than 4 years. We recommend the interval between two Pap should be 2-3 years, but not longer than 4 years between two Paps

P05-01

COMPARISON OF ANYPLEX II HPV HR AND HC2 TESTING IN A SCREENING POPULATION

A. Luyten, S. Strehlke, V. Weiß, K.U. Petry

Department for Gynecology, Obstetrics and Gynecologic oncology, Wolfsburg (Germany)

Background / Objectives

The pilot project WOLPHSCREEN (Wolfsburg primary HPV screening for better cervical cancer prevention) comprises 24,000 women aged 30+ years who attended for routine screening between 2006 and 2016. In July 2015 we tested samples from an HPV screening pilot project with Anyplex II HPV HR (Seegene Inc) for HPV genotypes to compare Anyplex performance with HC2 results as gold standard

Methods

The selected samples were originally collected and tested in 2007 with Hybrid Capture 2 (HC2). The original STM samples were stored at -22° Celsius. A first set of stored and fresh samples was tested in Wolfsburg earlier and showed that the 2007 samples were still of good quality.

On the 15th of July 2015 the central lab of Klinikum Wolfsburg received 292 samples for HPV testing with Anyplex. 282 samples were finally tested; eleven samples were excluded because of difficulties with the identification numbers. Out of this group another ten samples gave invalid results with HR-Anyplex, leaving 271 valid results.

Diagnoses are based on the maximum findings within long-term follow-up and are therefore more reliable than in cross-sectional trials.

Results

Overall 109 out of 116 HC2 positive CIN1+ tested positive for HPV high risk types with Anyplex (94%). More important, all of two invasive cancers, all three Adenocarcinoma in situ (AIS) and all 30 CIN3 cases tested positive for HPV-HR with Anyplex (35/35 = 100%). 4 out of 25 CIN2 and 3 out of 56 CIN1 were Anyplex negative but as CIN2 diagnoses are based on primary histology only, the overall sensitivity of the test for HSIL can be considered as excellent. Sensitivity and NPV for CIN3+ reached 100% in this cohort. 11 out of 150 HC2 negative samples tested positive for HR-HPV-types and 9

samples gave invalid results. If HC2 would be considered as benchmark, the specificity of Anyplex is 130/141 (92,2%).

The type distribution of the 14 individual HPV types resembled very much the distribution pattern found with LiPA genotyping in WOLPHSCREEN. HPV is the most frequent type in CIN3+ lesions but with less than 50% not as frequent as observed among young women at age 20-25 years in Wolfsburg (78-88%).

Conclusion

Anyplex II HPV HR showed an excellent sensitivity for CIN3+ and a plausible pattern of genotype distribution. The data is not sufficient for a reliable conclusion of the observed low specificity in HC2 negative samples.

P05-02

EVALUATION OF SEEGENE ANYPLEX II HPV GENOTYPING ASSAYS USING CERVICAL SAMPLES

S. Tabrizi, A. Cornall, M. Poljak, D. Machalek, S. Garland

The Royal Women's Hospital (Australia)

Background / Objectives

To compare the performance of Seegene Anyplex II HPV28 and Seegene Anyplex II HPVHR with EUROIMMUN EUROArray HPV (EuroArray), Roche Cobas® 4800 HPV assay (Cobas), Digene Hybrid Capture (HC) 2, Roche Linear Array (LA) and Roche Amplicor (AMP) for detection of high-risk HPV (HR-HPV) genotypes in women with high grade cervical cytology.

Methods

The Seegene HPV28 and HPVHR assays are multiplexed quantitative PCR melting-curve assays used for detection of 28 and 14 HPV genotypes, respectively, including 14 low-risk and 14 high-risk types. Seegene HPV28 is intended as a genotyping product for purposes of triage and patient follow-up tests, while Seegene HPVHR is intended as a primary cervical screening test. PreservCyt® specimens from women undergoing management of high-grade cytological abnormality were evaluated by these 2 assays. Concordance of detection of HR-HPV was calculated compared to previously tested EuroArray, Cobas, HC2, Amp, and LA HPV test results.

Results

Overall specimens were evaluated from 404 women with average age of 30 years. Specimens evaluated included 337 from women with >CIN2 and 67 from women with <CIN1 histological diagnosis. The concordance of HR-HPV detection with Seegene HPV28 compared to other HPV assays was 94.8% ($\kappa=0.844$) and 97.27% ($\kappa=0.911$) for EuroArray and LA; and with Seegene HPVHR 86.9.6% ($\kappa=0.640$), 96.53% ($\kappa=0.888$) and 96.8% ($\kappa=0.892$) for HC2, Cobas and Amp, respectively. Using HR-HPV detection for prediction of >CIN2 by Seegene HPV28 and HPVHR, sensitivity (90.18, 95%CI 86.48-93.14; 90.77, 95%CI 87.16-93.65) and specificity (67.16, 95%CI 54.60-78.15; 67.65, 95%CI 55.21-78.49) were not significantly different to the other HPV assays. Both assays had higher sensitivity for detection of \geq CIN2 than HC, and specificity of 94% (Seegene HPV28) and 95% (Seegene HPVHR) of HC in this high-risk population. Type-specific comparison between Seegene HPV28 and two full genotyping assays, EuroArray and LA, showed strong to perfect agreement ($\kappa\geq 0.800$) for most common genotypes.

Conclusion

The performance of Seegene HPV28 and HPVHR genotyping assays was highly concordant to other commercial HPV assays evaluated including EuroArray, HC2, Cobas, Amp and LA tests for detection of HR-HPV and prediction of \geq CIN2 in a high prevalence population. For full genotyping, Seegene performed well compared with LA and EuroArray, with some inter-assay variability, as would be expected.

P05-03

EVALUATION OF SAMPLE CELLULARITY AND NUCLEIC ACIDS STABILITY USING COPAN ENAT MEDIUM ASSOCIATED WITH FLOQSWABS: SELF-COLLECTED VERSUS CLINICIAN-VAGINAL SAMPLING.

M. Martinelli, R. Musumeci, C. Cocuzza

Department of Medicine and Surgery, University of Milano-Bicocca (Italy)

Background / Objectives

Vaginal self-sampling represents a promising alternative to increase women's participation to screening for HPV and sexually transmitted diseases. Clinical specimens for nucleic acids amplification tests (NAATs) are traditionally transported in viral or bacterial culture media. Copan developed eNAT™, a molecular medium designed for storage and transport of clinical samples for the detection infectious agents by NAATs, able to inactivate pathogens viability and preserve nucleic acids at room temperature for extended periods of time. The objectives of this study were to validate the performance of eNAT™ medium associated with FLOQSwabs™ (Copan, Brescia, Italy) for sample cellularity and nucleic acid stability in self- and clinician-collected vaginal samples.

Methods

Paired self-collected and physician administered vaginal samples using FLOQSwabs™ were randomly collected from 35 asymptomatic women attending the Cytology Unit, Synlab, Brescia, Italy. A further sample was also self-collected by all women at home. Samples were transported in eNAT™ medium and stored at -20°C until testing at the Microbiology Laboratory of the University Milano-Bicocca. Sample cellularity was evaluated, following nucleic acid extraction (NucliSENS® easyMAG, bioMérieux), by means of CCR5 gene and beta-actin mRNA quantification by real-time PCR as previously described^{1,2}. A separate sample aliquot was stored at -80°C and re-extracted and re-tested after 1 year to assess nucleic acid stability in eNAT™. Samples were also tested for the presence of HPV using AnyplexII HPV28 (Seegene).

Results

CCR5 and beta-actin copy numbers/2 ml eNAT™ median values were found to be 12275 and 169 x 10⁶; 12805 and 164 x 10⁶ respectively for home and point of care self-collected samples, for clinician-

collected samples values were 13333 and 165×10^6 respectively. Cellularity values after 1 year were found to be 14464 and 43×10^6 ; 12530 and 52×10^6 for home and point of care self-collected samples and 12971 and 64×10^6 for clinician-collected samples respectively. No statistical difference was found between sample cellularity in the different groups. HPV was detected in 26.5% (9/34) of women; 45% were positive in both samples, 33% only in self-collected and 22% only in clinician-collected samples.

Conclusion

Cellularity of both self- and clinician-collected vaginal samples using FLOQSwabs™ showed comparable results. Sample storage in eNAT™ medium for 1 year at -80°C showed good nucleic acid stability for both DNA and RNA. Data obtained demonstrated a good performance of both FLOQSwabs™ and eNAT™ medium in vaginal sample collection, transport and storage for NAATs.

References

1. F. Broccolo et al. J Clin Microbiol 2002; 4652-4658.
2. C. Jobin et al. J Immunol 1997; 158:226-234.

P06-01

HEALTH OUTCOME AND BUDGET IMPACT ANALYSIS OF A COMBINED CERVICAL SCREENING STRATEGY BASED ON PRIMARY HPV SCREENING AND TRIAGE WITH DUAL STAIN CYTOLOGY IN BELGIUM

K. Vandeweyer ¹, A. Van Roost ¹, K. Royackers ¹, A. Lefevre ¹, W.A. Tjalma ²

¹Roche Diagnostics Belgium, Vilvoorde, Belgium (Belgium), ²Multidisciplinary Breast Clinic - Unit Gynecologic Oncology, Antwerp University Hospital - University of Antwerp, Belgium (Belgium)

Background / Objectives

In EU countries with a Liquid Based Cytology (LBC) screening program cervical cancers are missed due to the low sensitivity of cytology. Primary HPV screening will hardly miss cancers due to the high sensitivity, but due to the lower specificity will lead to multiple unnecessary procedures (e.g. colposcopies, biopsies, follow-up visits).

To improve the outcome of a LBC screening program (every 3 years) a new algorithm was developed by the combination of primary HPV testing and dual stain cytology (CINtec® PLUS Cytology test (p16 & Ki-67)) (every 5 years). The objective of present study was to simulate the budget impact for both screening concepts and to compare the costs and health outcomes from a payer's and patient's perspective in Belgium.

Methods

A Budget Impact analysis based on a Markov model compared the costs and outcomes over two screening intervals (10 years compared to 6 years) for both screening algorithms. For the base case women aged between 30 and 65 years old were selected. For the Belgian situation it was assumed that all cervical intraepithelial neoplasia (CIN) were tested with dual staining. In the absence of intervention, disease may progress to another stage of disease, therefore patients with CIN 2 or higher were always sent to treatment. In our Markov model the probability of all disease transitions were based on the available literature (ATHENA and PALMS trials). For each screening algorithm, the total annual costs from the payer's perspective for \geq CIN2 and for cervical cancer were calculated.

Results

Compared to LBC primary screening, the use of cobas® HPV primary screening with triage by the dual stain cytology (CINtec® PLUS Cytology test) increases the detection of CIN2, CIN3 and cervical cancer by an average of 56%. The clinical impact of the algorithm showed a reduction in cervical cancer incidence of 30 % and a reduction in cancer death by 30 %. The budget impact analysis of the proposed screening algorithm showed a reduction of the screening budget by 22% (this is an annual cost saving of 5 million euro for the Belgian HealthCare system).

Conclusion

The strategy of primary HPV screening with a dual stain cytology triage reduces significantly the incidence of cervical cancer, cervical cancer death and the screening budget. The proposed screening algorithm is therefore beneficial for all stakeholders.

P07-01

PROLONGED ORAL CONTRACEPTIVES USE AND THE RISK OF ACQUISITION OF HUMAN PAPILLOMAVIRUS TYPE 16/18 INFECTIONS

I. Adhikari¹, H.M. Surcel², T. Luostarinen³, E. Pukkala³, D. Apter⁴, M. Lehtinen¹

¹University of Tampere (Finland), ²National Institute for Health and Welfare (THL) (Finland), ³Finnish Cancer Registry, Helsinki, Finland (Finland), ⁴Family Federation Finland, Helsinki, Finland (Finland)

Background / Objectives

To study the possible association between use of oral contraceptives and human papillomavirus infection.

Methods

We studied a cohort of female Helsinki University students (n=28,109) and other female university students (n=9,044) in the Helsinki area, born in 1946-1960 [1]. Our study material consists of 706 students (controls of an earlier breast cancer and oral contraceptives study) [1]. Linking the study material to the serum bank of the Finnish Maternity Cohort [2], we identified 297 women with stored first trimester serum samples. HPV type 16 and type 18 and Chlamydia trachomatis antibody analyses were done [3,4], and seropositivities used as measures of cumulative incidence of HPV16/18 and C. trachomatis infections. Data on oral contraceptives, smoking, parity, BMI and alcohol use were available for the cohort [1].

Results

Prolonged OC use for five years or more was non-significantly associated with increased risk for HPV16/18 seropositivity (odds ratio adjusted for age, smoking and C.trachomatis 2.6 with 95% confidence interval (0.8–8.5).

Conclusion

In conclusion, there is probably an association between the use of oral contraceptives and HPV16/18 infection. However, further studies using large scale follow up data are required to properly assess the association between OC use and the acquisition of HPV16/18 infection.

References

1. Hemminki, E., Luostarinen, T., Pukkala, E., Apter, D., Hakulinen, T. (2002). Oral contraceptive use before first birth and risk of breast cancer: a case control study. *BMC Womens Health*, 2, 9.
2. Koskela P, Anttila T, Bjørge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer* 2000;85(1):35-9.
3. Luostarinen T, af Geijersstam V, Bjorge T, et al. No excess risk of cervical carcinoma among women seropositive for both HPV16 and HPV6/11. *Int J Cancer* 1999;80(6):818-22.
4. Luostarinen T, Namujju PB, Merikukka M, et al. Order of HPV/Chlamydia infections and cervical high-grade precancer risk: a case-cohort study. *Int J Cancer* 2013;133(7):1756-9.

P07-02

CERVICAL MORBIDITY IN ALSACE, FRANCE. RESULTS FROM A REGIONAL ORGANIZED CERVICAL CANCER SCREENING PROGRAM

J.J. Baldauf¹, M. Fender², C. Bergeron³, M. Velten⁴, P. Pradat⁵, M. Arbyn⁶

¹Department of Obstetrics and Gynecology, CHU Hautepierre, Strasbourg, France (France), ²Association EVE, Illkirch Graffenstaden, France (France), ³Cerba Laboratory, Cergy Pontoise, France (France), ⁴Department of Epidemiology and Public Health, Bas-Rhin cancer Registry, University of Strasbourg, Strasbourg, France (France), ⁵Department of Hepatology, Croix-Rousse Hospital, Hospices Civils de Lyon, Lyon, France (France), ⁶Belgian Cancer Centre/Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium (France)

Background / Objectives

In 1994, a pilot program of cervical cancer screening was introduced in the Alsace region, France. The objectives of this program are to increase screening coverage and to insure high quality in every step of the screening process. Within this context, we assessed cervical morbidity in Alsace before the HPV vaccinated population reaches the age of screening.

Methods

Data on cervical lesions and cancers were collected for the period between September 1st, 2008 and August 31st, 2011. The data collection covers the Alsace region which comprises the Bas-Rhin and the Haut-Rhin departments for a total of about 500,000 women aged 25 to 65 years. The screening relies on existing medical structures and collects data from all cytopathology laboratories in the area. Data are centralized and managed by the EVE association. Cytological and histological data are completed with data from two cancer registries of the Alsace region.

Results

During the 2008-2011 period, 565,153 smears were performed in women aged 25 to 64 years in Alsace, representing an average of 1.1 smear per woman and 1.62 smear/screened woman. The overall screening coverage was 70.1% over the 3-year period and varied according to age. It increased from 63.4% in women aged 25-29 to 83.4% in women aged 30-34 and then decreased regularly to 56.7% in 60-64-year-old women. Over the same period, 2,664 cases of CIN1, 962 CIN2

and 1,283 CIN3 were reported representing a prevalence of 1.78/1,000, 0.64/1,000 and 0.86/1,000 screened women, respectively. The world standardized prevalence of CIN1, CIN2, and CIN3 with its 95% CI was 2.03/1,000 [1.97-2.1/1,000], 0.73/1,000 [0.69-0.77/1,000] and 0.97/1,000 [0.93-1.03/1,000], respectively. Moreover, 154 cervical cancers were reported giving an incidence proportion of 10.3/100,000 women-years. The incidence increased from 5.6/100,000 in women aged 25 to 29 years to about 13.3/100,000 in women between 40 and 54 years, and decreased after 55 years reaching 7.5/100,000 in 60-64 year-old women.

Conclusion

The Alsace region is the first French region where an organized cervical cancer screening program was implemented. The overall screening coverage of 70% at three years is higher than the national rate (57%) and reflects the strength of an organized screening program. The EVE database will be a useful tool to assess trends in cervical morbidity over time and to further assess the impact of screening as well as of HPV vaccination.

P07-03

PREVALENCE OF HPV INFECTION AMONG ADOLESCENT GIRLS IN MOSCOW REGION, RUSSIAN FEDERATION

V. Krasnopolskij, N. Zarochentseva, J. Belaya, E. Bulycheva

The Scientific Research Institute of Gynecology and Obstetrics of Moscow Region, Moscow, Russian Federation. (Russian federation)

Background / Objectives

The human papillomavirus (HPV) is the important risk factor of cervical and other anogenital cancers in woman. Two prophylactic vaccines have been provided for prevention of HPV-associated cancers since 2007 in many countries. The objective of this study was to assess the prevalence of HPV in 14-17 aged girls in Moscow region.

Methods

640 girls from 14 to 17 years of age from schools and colleges of Moscow region were examined in polyclinics during annual prophylactic medical examination. For additional gynecological examination consent forms were signed by parents of girls. Cervical specimens were collected and tested for 14 types of HPV by using AmpliSens®PCR Kits (12 high risk HPV types and HPV 6/11).

Results

Among all 640 girls 176 (27,5%) were sexually active. Most of them (80,3%) were students of colleges vs 19,7% girls from schools. Among schoolgirls 13,5% (n=7) had become sexually active at <15 years of age, 20,8% (n=53) at 15-16 years of age, 35,2% (n=116) at 16-17 years of age. The prevalence of high risk HPV types (HR HPV) among all girls was 17% while among sexually active adolescents was observed 50,5%. 19,8% samples from sexually active adolescents were positive for HR HPV 16/18 and 3,0% for LR HPV 6/11. Interestingly, 4,5% of sexually naive girls were HR HPV positive.

Conclusion

Our study confirms high prevalence of human papillomavirus soon after sexual debut of adolescent girls. These findings support early age of vaccination as implemented by most public health programs to maximize effectiveness of HPV vaccination strategies.

P07-04

HPV AND BREAST CANCER...IS THERE A RELATIONSHIP?

S. Delgado García¹, J.C. Martínez Escoriza¹, A. Alba², T.A. Martín Bayón¹, H. Ballester Galiana¹, G. Peiró¹, P. Caballero³, J. Ponce Lorenzo³

¹University General Hospital of Alicante (Spain), ²Director of Genetic Departament. Instituto de Estudios Celulares y Moleculares, Lugo (Spain), ³University of Alicante (Spain)

Background / Objectives

Human Papillomavirus (HPV) has been detected in several types of cancers such as cervix, vulva, penis, oral cavity or esophagus. There are controversial reports on the role of HPV in some breast cancer around the world. Since 1992, some studies detected HPV-DNA in human breast cancer (1-38); however some authors had reported negative results (39-48). Furthermore, the studies are very heterogeneous in terms of the methodology employed. The aim of this study was to explore the presence of HPV-DNA in a case-control study, in a series of samples obtained from breast surgeries at the University General Hospital of Alicante (Spain), estimating the strength of the association between the presence of HPV in benign breast disease and breast cancer.

Methods

A case-control study was performed to evaluate the presence of HPV infection in a subset of 250 embedded breast cancer, as cases, and 250 embedded benign breast diseases, as controls. The estimated exposure rate (presence of HPV) was 25% in the cases, and 14% in the controls, with a confidence level of 95% and a statistical power of 85% in detecting OR >2. The search for viral DNA was carried out at the Instituto de Estudios Celulares y Moleculares (Spain). Sections measuring 10 µm in thickness were obtained from the tumor area of the paraffin block for the identification of viral DNA. The samples were subjected to three different HPV detection and genotyping methods: GP5+/GP6+ consensus primers, CLART® HPV2 amplification kit (Genomica) and HPV Direct Flow CHIP kit (Master Diagnostica).

Results

The final study included 437 samples: 251 cases (57.4%) and 186 controls (42.6%). The data obtained for determining the presence of HPV in breast cancer tissue samples and establishing the comparisons with the samples corresponding to benign breast tissue, were analyzed using the Chi-

squared test. In this regard, the HPV exposure rate among the cases was significantly higher (51.8%) than the HPV exposure rate in the controls (26.3%) ($p < 0.001$). The raw odds ratio was 3.0 (CI 95%: 2.0-4.5). On applying the binary logistic regression model to control for confounding variables, the OR assigned to HPV was seen to be 4.034 (CI 95%: 2.213-7.352), which means a higher risk of suffering cancer in the presence of HPV, taking into account patient age and breastfeeding. HPV-16 was the most frequent.

Conclusion

HPV is present in a subset of breast carcinomas and therefore, potentially pathogenic related. Moreover, taking in consideration age and breastfeeding, reinforce the influence of HPV-positivity in determining whether a given patient belongs to the case or control group. Nevertheless, further research is necessary to confirm our results.

References

- (1) Joshi D, Buehring GC. Are viruses associated with human breast cancer? Scrutinizing the molecular evidence. *Breast Cancer Res Treat* 2012;135(1):1-15.
- (2) Alibek K, Kakpenova A, Mussabekova A, Sypabekova M, Karatayeva N. Role of viruses in the development of breast cancer. *Infect Agent Cancer* 2013;8(1):32-37.
- (3) Hedau S, Kumar U, Hussain S, Shukla S, Pande S, Jain N, et al. Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women. *BMC Cancer* 2011;11(1):27.
- (4) Simoes PW, Medeiros LR, Simoes Pires PD, Edelweiss MI, Rosa DD, Silva FR, et al. Prevalence of human papillomavirus in breast cancer: a systematic review. *Int J Gynecol Cancer* 2012;22(3):343-347.
- (5) Frega A, Lorenzon L, Bononi M, De Cesare A, Ciardi A, Lombardi D, et al. Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer. *Eur J Gynaecol Oncol* 2012;33(2):164-167.
- (6) Li N, Bi X, Zhang Y, Zhao P, Zheng T, Dai M. Human papillomavirus infection and sporadic breast carcinoma risk: a meta-analysis. *Breast Cancer Res Treat* 2011;126(2):515-520.
- (7) Di Lonardo A, Venuti A, Marcante ML. Human papillomavirus in breast cancer. *Breast Cancer Res Treat* 1992;21(2):95-100.
- (8) Hennig EM, Suo Z, Thoresen S, Holm R, Kvinnsland S, Nesland JM. Human papillomavirus 16 in breast cancer of women treated for high grade cervical intraepithelial neoplasia (CIN III). *Breast Cancer Res Treat* 1999;53(2):121-135.

- (9) Yu Y, Morimoto T, Sasa M, Okazaki K, Harada Y, Fujiwara T, et al. HPV33 DNA in premalignant and malignant breast lesions in Chinese and Japanese populations. *Anticancer Res* 1999;19(6B):5057-5061.
- (10) Yu Y, Morimoto T, Sasa M, Okazaki K, Harada Y, Fujiwara T, et al. Human papillomavirus type 33 DNA in breast cancer in Chinese. *Breast Cancer* 2000;7(1):33-36.
- (11) Liu Y, Klimberg VS, Andrews NR, Hicks CR, Peng H, Chiriva-Internati M, et al. Human papillomavirus DNA is present in a subset of unselected breast cancers. *J Hum Virol* 2001;4(6):329-334.
- (12) Damin AP, Karam R, Zettler CG, Caleffi M, Alexandre CO. Evidence for an association of human papillomavirus and breast carcinomas. *Breast Cancer Res Treat* 2004;84(2):131-137.
- (13) Noguera C, Piuze V, ML-Soto de Ferrini S, Acosta P, Cerdera Noguera A. Virus Papiloma Humano y su Relación con Cáncer de Mama. Informe Preliminar. 2004 Disponible en: <http://www.unne.edu.ar/unnevieja/Web/cyt/com2004/3-Medicina/M-082.pdf>
- (14) Widschwendter A, Brunhuber T, Wiedemair A, Mueller-Holzner E, Marth C. Detection of human papillomavirus DNA in breast cancer of patients with cervical cancer history. *Journal of Clinical Virology* 2004;31(4):292-297.
- (15) Kan C, Iacopetta B, Lawson J, Whitaker N. Identification of human papillomavirus DNA gene sequences in human breast cancer. *Br J Cancer* 2005;93(8):946-948.
- (16) Kroupis C, Markou A, Vourlidis N, Dionyssiou-Asteriou A, Lianidou ES. Presence of high-risk human papillomavirus sequences in breast cancer tissues and association with histopathological characteristics. *Clin Biochem* 2006;39(7):727-731.
- (17) Yasmeeen A, Bismar TA, Kandouz M, Foulkes WD, Desprez P, Al Moustafa A. E6/E7 of HPV type 16 promotes cell invasion and metastasis of human breast cancer cells. *Cell cycle* 2007;6(16):2038-2042.
- (18) Choi YL, Cho EY, Kim JH, Nam SJ, Oh YL, Song SY, et al. Detection of human papillomavirus DNA by DNA chip in breast carcinomas of Korean women. *Tumour Biol* 2007;28(6):327-332.
- (19) Akil N, Yasmeeen A, Kassab A, Ghabreau L, Darnel A, Al Moustafa A. High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study. *Br J Cancer* 2008;99(3):404-407.
- (20) Duò D, Ghimenti C, Migliora P, Pavanelli MC, Mastracci L, Angeli G. Identification and characterization of human papillomavirus DNA sequences in Italian breast cancer patients by PCR and line probe assay reverse hybridization. *Molecular medicine reports* 2008;1(5):673-677.
- (21) Khan N, Castillo A, Koriyama C, Kijima Y, Umekita Y, Ohi Y, et al. Human papillomavirus detected in female breast carcinomas in Japan. *Br J Cancer* 2008;99(3):408-414.

- (22) Aceto GM, Solano AR, Neuman MI, Veschi S, Morgano A, Malatesta S, et al. High-risk human papilloma virus infection, tumor pathophenotypes, and BRCA1/2 and TP53 status in juvenile breast cancer. *Breast Cancer Res Treat* 2010;122(3):671-683.
- (23) De Villiers E, Sandstrom RE, Zur Hausen H, Buck CE. Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast. *Breast Cancer Res* 2005;7(1):R1-11.
- (24) de Leon DC, Montiel DP, Nemcova J, Mykyskova I, Turcios E, Villavicencio V, et al. Human papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients. *BMC Cancer* 2009 Jan 22;9:26.
- (25) Mendizabal-Ruiz A, Morales J, Ramirez-Jirano L, Padilla-Rosas M, Morán-Moguel M, Montoya-Fuentes H. Low frequency of human papillomavirus DNA in breast cancer tissue. *Breast Cancer Res Treat* 2009;114(1):189-194.
- (26) He Q, Zhang SQ, Chu YL, Jia XL, Wang XL. The correlations between HPV16 infection and expressions of c-erbB-2 and bcl-2 in breast carcinoma. *Mol Biol Rep* 2009;36(4):807-812.
- (27) Heng B, Glenn W, Ye Y, Tran B, Delprado W, Lutze-Mann L, et al. Human papilloma virus is associated with breast cancer. *Br J Cancer* 2009;101(8):1345-1350.
- (28) Yavuzer D, Salepci T, Karadayi N, Baloglu H, Kucukodaci Z. Human papillomavirus is not associated with breast carcinoma. *Breast Cancer Res Treat* 2010;122(3):899-900.
- (29) Glenn WK, Whitaker NJ, Lawson JS. High risk human papillomavirus and Epstein Barr virus in human breast milk. *BMC Res Notes* 2012;5:477-0500-5-477.
- (30) Manzouri L, Salehi R, Shariatpanahi S. Prevalence of human papilloma virus among women with breast cancer since 2005-2009 in Isfahan. *Advanced biomedical research* 2014;3:75.
- (31) Ohba K, Ichiyama K, Yajima M, Gemma N, Nikaido M, Wu Q, et al. In vivo and in vitro studies suggest a possible involvement of HPV infection in the early stage of breast carcinogenesis via APOBEC3B induction. *PLoS One* 2014 May 23;9(5):e97787.
- (32) Lv YR, Wang JL, Zhang K, Gao HD, Sun JZ, Gong YY, et al. Human papilloma viruses (HPVs) no co-existence in breast cancer and cervical cells in the same patient. *Chin J Physiol* 2014;57(2):105-106.
- (33) Piana A, Sotgiu G, Muroni M, Cossu-Rocca P, Castiglia P, De Miglio M. HPV infection and triple-negative breast cancers: an Italian case-control study. *Virol J* 2014;11(1):190.
- (34) Wang T, Zeng X, Li W, Zhu H, Wang G, Liu X, et al. Detection and analysis of human papillomavirus (HPV) DNA in breast cancer patients by an effective method of HPV capture. *PLoS One* 2014;9(3):e90343.
- (35) Fernandes A, Bianchi G, Feltri AP, Pérez M, Correnti M. Presence of human papillomavirus in breast cancer and its association with prognostic factors. *ecancermedicalscience* 2015;9: 548.

- (36) Fu L, Wang D, Shah W, Wang Y, Zhang G, He J. Association of human papillomavirus type 58 with breast cancer in shaanxi province of China. *J Med Virol* 2015; 87(6):1034-40.
- (37) Li J, Ding J, Zhai K. Detection of Human Papillomavirus DNA in Patients with Breast Tumor in China. *PLoS one* 2015;10(8):e0136050.
- (38) Salehpour M, Tayyebi Meibodi N, Teimourpour R, Ghorani-Azam A, Sepahi S, Rostami S, et al. Frequency of Human Papillomavirus Genotypes 6, 11, 16, 18 And 31 in Paraffin-Embedded Tissue Samples of Invasive Breast Carcinoma, North-East of Iran. *Iranian Journal of Pathology* 2015;10(3):192-198.
- (39) Bratthauer G, Tavassoli F, O'Leary T. Etiology of breast carcinoma: no apparent role for papillomavirus types 6/11/16/18. *Pathology-Research and Practice* 1992;188(3):384-386.
- (40) de Cremoux P, Thioux M, Lebigot I, Sigal-Zafrani B, Salmon R, Sastre-Garau X. No evidence of human papillomavirus DNA sequences in invasive breast carcinoma. *Breast Cancer Res Treat* 2008;109(1):55-58.
- (41) Wrede D, Luqmani Y, Coombes R, Vousden K. Absence of HPV 16 and 18 DNA in breast cancer. *Br J Cancer* 1992;65(6):891.
- (42) Czerwenka K, Heuss F, Hosmann JW, Manavi M, Lu Y, Jelincic D, et al. Human papilloma virus DNA: a factor in the pathogenesis of mammary Paget's disease? *Breast Cancer Res Treat* 1996;41(1):51-57.
- (43) Gopalkrishna V, Singh U, Sodhani P, Sharma J, Hedau S, Mandal A, et al. Absence of human papillomavirus DNA in breast cancer as revealed by polymerase chain reaction. *Breast Cancer Res Treat* 1996;39(2):197-202.
- (44) Lindel K, Forster A, Altermatt HJ, Greiner R, Gruber G. Breast cancer and human papillomavirus (HPV) infection: no evidence of a viral etiology in a group of Swiss women. *The breast* 2007;16(2):172-177.
- (45) Hachana M, Ziadi S, Amara K, Toumi I, Korbi S, Trimeche M. No evidence of human papillomavirus DNA in breast carcinoma in Tunisian patients. *The Breast* 2010;19(6):541-544.
- (46) Silva Jr RG, da Silva BB. No evidence for an association of human papillomavirus and breast carcinoma. *Breast Cancer Res Treat* 2011;125(1):261-264.
- (47) Chang P, Wang T, Yao Q, Lv Y, Zhang J, Guo W, et al. Absence of human papillomavirus in patients with breast cancer in north-west China. *Medical Oncology* 2012;29(2):521-525.
- (48) Vernet-Tomas M, Mena M, Alemany L, Bravo I, De Sanjose S, Nicolau P, et al. Human papillomavirus and breast cancer: no evidence of association in a spanish set of cases. *Anticancer Res* 2015;35(2):851-856.

P07-05

EPIDEMIOLOGY OF HOSPITALIZATIONS RELATED TO CERVICAL CANCER IN SPAIN (2009-2013).

R. Gil-Prieto¹, R. Pascual-Garcia¹, M. San-Martin², N. Lopez², A. Gil-De Miguel¹

¹Area of Preventive Medicine and Public Health. Rey Juan Carlos University. Madrid (Spain), ²Medical Department. Sanofi Pasteur MSD (Spain)

Background / Objectives

This epidemiological survey was undertaken to estimate the burden of hospitalization related to cervical cancer in Spain during a five year period (2009-2013).

Methods

Retrospective survey by reviewing data of the National Surveillance System for Hospital Data (Conjunto Mínimo Básico de Datos), including more than 98% of Spanish hospitals. All hospitalizations related to malignant neoplasm of cervix uteri or carcinoma in situ of cervix uteri, reported during 2009-2013 period, were analysed. Codes for cervical cancer and carcinoma in situ were selected by using the 9th International Classification of Diseases: ICD-9-CM 180, 180.0, 180.1, 180.8, 180.9 y 233.1. The annual incidence of hospitalization, average length of hospitalization and in-hospital case-fatality rate were calculated using municipal register data.

Results

A total of 30,749 hospital discharges for cervical cancer were reported during the study period. Of those, 10,460 were coded as carcinoma in situ (CIS), corresponding to 9,389 women (1,1 hospitalizations per woman) and 20,527 were coded as malignant neoplasm (MN), corresponding to 11,864 women, (1,7 hospitalizations per woman). CIS was coded as main cause of hospitalization in the 86,30% of the registers (9,022), versus only 40,38% of the MN (12,416). The cause of hospitalization for the remaining 60% of the patients was related to the MN- i.e. chemotherapy, haemorrhages-. Mean age of hospitalization was 42,14 years old (SD=12,13) in CIS and 54,76 years old (SD=14,86) in MN and significantly increased in the study period. Average length of hospitalization was 3,07 (SD 4,21) and 7,85 (SD 10,33) days, for CIS and MN respectively.

The annual incidence of hospitalizations was 8,96 per 100,000 (CI95%: 8,79-9,13) for CIS and 17,57 per 100,000 (CI95%: 17,33-17,81) for MN, reaching the maximum rate -17,98 per 100,000 (CI95%:

17,49-18,47)- in the 30-44 year old group in CIS and 33,40 per 100,000 (CI95%: 32,66-34,15) in the 45-59 year old group in MN.

In-hospital case-fatality rate was 0,22% (CI95%: 0,13-0,31) for CIS and 7,95% (CI95%: 7,58-8,32) for MN.

Almost all the hospitalizations had at least one procedure associated. Most frequent procedures were conization, cauterization, salpingoophorectomy, hysterectomy, radiotherapy and blood transfusions.

Conclusion

Hospitalizations related to cervical cancer pose a significant health threat in Spain with an important number of medical and surgical procedures and an important re-admission rate.

P07-06

HPV-RELATED CANCER IN NORWAY: SECULAR INCIDENCE TRENDS AND PREVENTIVE POTENTIAL OF HPV VACCINATION

B.T. Hansen, S. Campbell, M. Nygård

Department of Research, Cancer Registry of Norway (Norway)

Background / Objectives

Assessment of the HPV-related cancer burden at cervical as well as non-cervical sites is needed to estimate the cancer preventive potential of HPV vaccination.

The objectives of the present study are: A) To describe incidence trends in cancer of the cervix, vulva, vagina, anus, penis and oropharynx in Norway during the period 1953-2014. B) To quantify the cancer preventive potential of HPV vaccination in Norway.

Methods

We present annual age-adjusted rates of primary squamous cell cancers of the cervix, vulva, vagina, anus, penis and oropharynx, and adenocarcinoma of the cervix, in Norway for the period 1953-2014. The data was collected from the Cancer Registry of Norway. To estimate the cancer preventive potential of HPV vaccination, the current cancer burden at each site was multiplied with the respective fraction of cancer cases attributable to any HPV, and to HPV16/18. Attributable fractions for each cancer site were derived from epidemiology literature reviews.

Results

Increasing cancer trends were observed for some of the HPV-related cancer sites. Currently, for all sites combined, 480 new cancer cases per year can be attributed to HPV in Norway, of which 371 can be attributed to HPV16/18.

Conclusion

These data demonstrate that HPV vaccination may have a large public health impact in Norway. Moreover, the incidence of some HPV-related cancers is increasing, which adds to the importance of HPV vaccination for reducing the cancer burden in the future.

P07-07

CERVICAL CANCER INCIDENCE AND MORTALITY IN THE RUSSIAN FEDERATION

P. Lopukhov¹, N. Briko¹, A. Khaldin², S. Kharit³, O. Zhukova², D. Isaeva², O. Lupashko²

¹I.M. Sechenov First Moscow State Medical University, Moscow (Russian federation), ²Moscow Research and Practical Center of Dermatology, Venereology and Cosmetology (MRPCDVC), Moscow (Russian federation), ³The Scientific Research Institute of Children's Infections, S-Petersburg (Russian federation)

Background / Objectives

Cervical cancer is one of the most common cancers in women and incidence of cervical cancer has increased in younger generations of women most likely as a result of changing sexual behavior and high prevalence of HPV infection in population. Almost all cervical cancer cases are attributable to HPV. Our aim was to analyze the cervical cancer incidence and mortality in the Russian Federation and in Moscow during 8-years period and describe potential reasons of trends.

Methods

We analyzed retrospective data with diagnostic code related to cervical cancer from the official statistic of cancer register using age-standardized (world standard) incidence and mortality rates between January 2007 and December 2014 in the Russian Federation, in Moscow, and in other regions of the country.

Results

The overall number of new cervical cancer cases during 8 years period in Moscow was 6 948 (average 869,5 cases per year), in Russia – 177 738 new cases (average 14 717 cases per year). Age-standardized incidence rates of cervical cancer in Moscow had gradually declined from 10,3 per 100 000 of women in 2007 to 8,0 in 2014 while on country level age-standardized incidence rates of cervical cancer had increased from 12,5 per 100 000 of women in 2007 to 14,5 in 2014. In 2007–2014 the regions of Russia with the highest cervical cancer incidence (in median) were: Zabaykalsky Krai – 28,7 per 100 000, the Republic of Karelia – 26,1 per 100 000, the Republic of Buryatia – 22,4 per 100 000.

Average age of new patients with diagnosis cervical cancer slowly decreasing (the patients “become younger”), it had changed from 56 years in 1997 to 52 years in 2014.

Age-standardized cervical cancer mortality rates in Moscow had gradually declined from 4,3 in 2007 per 100 000 of women to 3,9 in 2014. In the whole of Russia age-standardized cervical cancer mortality rates ranged from 5,0 to 5,4 per 100 000 of women, with no trend to decline.

Conclusion

The cervical cancer incidence and mortality in Moscow is lower than in Russia. There are two opposite trends for cervical cancer incidence in Moscow and in Russia: incidence rate is significantly decrease in the Moscow while increasing in Russia. The positive trend in Moscow can be associated with higher level of healthcare system and socioeconomic status of the capital city. Introduction of mass prevention strategies including HPV vaccination and HPV screening can significantly reduce cervical cancer incidence and mortality in the country.

P07-08

Cervical screening history and rare histological types of invasive cervical cancer: a population-based nested case-control study in Sweden

J. Lei¹, B. Andrae², J. Wang¹, S.N. Kleppe¹, M. Elfström¹, P. Sparén¹

¹Karolinska Institutet (Sweden), ²Karolinska Institutet; Uppsala University/Region of Gävleborg (Sweden)

Background / Objectives

The effectiveness of cervical screening on cervical cancer has been thoroughly evaluated for squamous cell carcinoma and to some extent also for adenocarcinoma of the cervix^{1,2}. However, no studies regarding screening effectiveness have been conducted for rare histological types of invasive cervical cancer, which are considered more aggressive³⁻⁵.

Methods

We conducted a population-based, age matched case-control study, nested within a cohort of Swedish women born 1909-1986 followed 2002-2011. 284 cases of rare histological types of cervical cancer were identified; adenosquamous carcinoma (ASC), glassy cell carcinoma (GCC), clear cell carcinoma (CCC), cervical neuroendocrine carcinomas (CNECs), small cell carcinoma (SmCC) and undifferentiated carcinoma (UC).

Results

Screening within the recommended interval was associated with a lower risk of rare histological types of cervical cancer (IRR:0.45, CI: 0.34-0.59; ASC IRR:0.43, CI:0.30-0.63, GCC IRR:0.57, CI: 0.19-1.70, CCC IRR:0.22, CI: 0.08-0.60, and CNECs/SmCC/UC IRR: 0.55, CI: 0.32-0.93). Cervical screening was particularly effective for women age 30-49 (IRR: 0.40, CI: 0.25-0.63) and 50-65 (IRR:0.26, CI: 0.16-0.42). Screening was also effective in preventing advanced cancers (IRR: 0.31, CI: 0.20-0.47) and led to diagnosis at an earlier stage for invasive cancer, especially for young women.

Conclusion

Cervical screening with cytology can significantly reduce the risk of rare histological types of invasive cervical cancer, and downstaging also reduced the proportion of advanced cancer. Further research

comparing different screening strategies with HPV and/or cytology is needed to further optimize the prevention of rare histological types of cervical cancer.

References

1. IARC, IARC handbooks of cancer prevention. Cervix cancer screening. Vol. 10. 2005, Lyon, France: International Agency for Research on Cancer Press.
2. Andrae, B., et al., Screening-preventable cervical cancer risks: evidence from a nationwide audit in Sweden. *J Natl Cancer Inst*, 2008. 100(9): p. 622-9.
3. Chen, J., O.K. Macdonald, and D.K. Gaffney, Incidence, mortality, and prognostic factors of small cell carcinoma of the cervix. *Obstet Gynecol*, 2008. 111(6): p. 1394-402.
4. Satoh, T., et al., Gynecologic Cancer InterGroup (GCIg) consensus review for small cell carcinoma of the cervix. *Int J Gynecol Cancer*, 2014. 24(9 Suppl 3): p. S102-8.
5. Hasegawa, K., et al., Gynecologic Cancer InterGroup (GCIg) consensus review for clear cell carcinoma of the uterine corpus and cervix. *Int J Gynecol Cancer*, 2014. 24(9 Suppl 3): p. S90-5.

(Selected reference)

P07-09

SEROPREVALENCE OF 19 HUMAN PAPILLOMAVIRUS (HPV) TYPES IN A NATIONWIDE REPRESENTATIVE CROSS-SECTIONAL SURVEY IN GERMANY IN THE PRE-VACCINE ERA

A. Loenenbach¹, C. Poethko-Müller², C. Renschmidt¹, M. Thamm², T. Harder¹, M. Pawlita³, T. Waterboer³, J. Schröter³, Y. Deléré¹, O. Wichmann¹, M. Wiese-Posselt¹

¹Department for Infectious Disease Epidemiology, Robert Koch Institute (RKI), Berlin/Germany (Germany), ²Department of Epidemiology and Health Monitoring, RKI, Berlin/Germany (Germany), ³Division of Molecular Diagnostics of Oncogenic Infections, German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ), Heidelberg/Germany (Germany)

Background / Objectives

There is only limited data on the distribution of human papillomavirus (HPV) in Germany. We examined nationally representative data on men and women aged 17-79 years to determine the seroprevalence and distribution of 19 HPV types in 1997 to 1999, before the introduction of HPV vaccines in Germany.

Methods

The "German National Health Interview and Examination Survey 1998" was carried out by the Robert Koch Institute from 1997 to 1999. A total of 7,124 subjects – a representative sample of the residential population aged 17-79 years – were interviewed and medically examined. Final analyses for HPV seroprevalence included sera and survey data from to date 6,038 subjects. The sera were tested against antibodies to the capsid protein L1 by multiplex serology at the German Cancer Research Center (DKFZ), Heidelberg (HPV types: mucosal high risk (HR): 16, 18, 31, 33, 35, 39, 45, 52, 58, 59; mucosal low risk (LR): 6, 11, cutaneous: 1, 4, 8, 10, 38, 41, 49). HPV seroprevalence was used as a marker of cumulative HPV infections. Previously established HPV type-specific cut-off values were applied for defining HPV seropositivity (1, 2). Weighted data were used to describe seroprevalence stratified by sex and age.

Results

In the survey sample, antibody seroprevalence for at least one of the 19 types analyzed was 73% (95% confidence interval (CI) 71-75%) in women and 74% (95% CI 72-76%) in men. The antibody prevalence of mucosal types was 35% (95% CI 33-37%) in women and 32% (95% CI 30-34%) in men. Antibody prevalence of cutaneous types was 63% (95% CI 61-65%) in women and 65% (95% CI 62-67%) in men. Nine percent (95% CI 8-10%) of the participating women and 5% (95% CI 4-6%) of men were seropositive for the high-risk type HPV 16. Antibody prevalence in vaccine-relevant types regarding the new nonavalent vaccine (HPV types: 6, 11, 16, 18, 31, 33, 52, 58, 45) was 27% (95% CI 24-28%) in women and 23% (95% CI 21-24%) in men. Seroprevalence of any of the nonavalent vaccine types increased with age and peaked in women and men in the 35-49 age groups. A second peak was found in women in the 60-64 age group.

Conclusion

Representative data on the antibody response to HPV is scarce. We assessed the HPV seroprevalence among adult participants aged 17-79 years of a nationwide representative cross-sectional survey. In Germany infections by vaccine-preventable HPV types were highly prevalent before the introduction of HPV vaccines. Our data on HPV seroprevalence in the pre-vaccine era are crucial for the evaluation of the existing HPV vaccination recommendation in Germany.

References

1. Clifford GM, Shin HR, Oh JK, Waterboer T, Ju YH, Vaccarella S, et al. Serologic response to oncogenic human papillomavirus types in male and female university students in Busan, South Korea. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2007;16(9):1874-9.
2. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, et al. Seroprevalence of 34 human papillomavirus types in the German general population. *PLoS Pathog.* 2008;4(6):e1000091.

P07-10

HPV PREVALENCE AND TYPE-DISTRIBUTION IN CORRELATION WITH AGE AND CERVICAL CYTOLOGIC ABNORMALITIES IN CROATIAN FEMALE POPULATION

T. Marijan, S. Ljubin-Sternak, V. Ticic, J. Vranes

Andrija Stampar Teaching Institute of Public Health, Department of Clinical Microbiology, Zagreb, Croatia (Croatia)

Background / Objectives

The aim of this study was to determine the prevalence and the type-distribution of high-risk HPV (hrHPV) genotypes according to age and severity of cervical lesion in female population of Zagreb region.

Methods

The study included all women, who were during the eight-month period (December 2013-July 2014), on their gynaecologist's request, tested on hrHPV infection with the real-time PCR test (Cobas HPV Test). The examinees were divided according to age in two main groups (<30 years and ≥30 years) and according to the severity of cervical lesion, based on Pap test result, in two groups (ASCUS/CIN1 as LSIL and CIN2/CIN3 as HSIL). Differences between observed groups were compared by using Fisher's exact test with p value <0.01 considered statistically significant.

Results

Out of the 3542 women tested, hrHPV infection was detected in 39.7% (52.8% in women younger than 30 years and 32.9% in the age group ≥30 years; p<0.01). All detected types (HPV16, HPV 18 and the group of other twelve hrHPV types - 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) were more common in younger women, especially in teenagers, where the prevalence of hrHPV infection was as high as 68.8%. In 749 women with LSIL the hrHPV infection was detected in 41.9%, while out of 363 women with HSIL 61.2% were hrHPV positive (p<0.01). The portion of HPV 16 significantly increased with severity of cervical lesion (28.3% in LSIL, 40.1% in HSIL; p<0.01), while the portion of the group of other hrHPV types was more common in LSIL (82.8%) than in HSIL (71.2%) (p<0.01). In women ≥30 years old infection with HPV 16 was significantly more common as single-type (69.6%) compared to women younger than 30 years where single-type infection with HPV 16 was detected in 37.5%

($p < 0.01$). The same was observed for HPV 18 (57.4% in older and 31.3% in women younger than 30 years; $p < 0.01$).

Conclusion

This study confirms existing data about higher HPV prevalence rates as well as predominance of multiple HPV infections in younger age groups. Furthermore, the prevalence of HPV 16, as expected, was higher in more severe cervical lesions.

P07-11

HR HPV VIRUS PRESENCE IN WOMEN - COVERED BY NATIONAL SCREENING PROGRAM FOR CERVICAL CARCINOMA IN POLAND

W. Olszewski¹, M. Rekosz¹, J. Faryna¹, A. Prusaczyk², A. Dansonka-Mieszkowska¹, J. Moes-Sosnowska¹, P. Wisniewski¹, B. Janik¹

¹Department of Pathology and Diagnostic Laboratory, Oncology Center, Warsaw (Poland), ²Center for Medical Diagnostics, Siedlce (Poland)

Background / Objectives

The purposes of the study:

1. Establishing of population frequency of HR HPV infection was the main purpose of the study concerning cervical carcinoma screening program.
2. Logistics for co-test (cytology and HR HPV test) implementation in Poland.

Methods

The study covered unselected 10112 women age 30-59 years old, including 3089 from urban and 7023 from rural area. All samples were genotyped with Abbott High-Risk HPV Real-time PCR on automated platform Abbott M2000. Conventional Pap tests were performed the same time.

Results

In the studied population generally there were 6,6% positive tests for HR HPV (7,5% in urban area and 4,9% in rural population). Among positive cases type HPV16 prevailed followed by type 18. Percentage of HR HPV positive cases was decreasing with age.

Correlation of HR HPV test results with cytological reports revealed that only in 27% HR HPV positive cases cytological abnormality (LGSIL, ASCUS, ASCUS-H, HGSIL) were found.

Conclusion

The percentage of HR HPV case in Poland was relatively low, especially in rural population. The project allowed preparing introduction of co-test in national prophylactic program for cervical carcinoma.

P07-12

Anal High Risk HVP: Gender Comparison For Detection In Puerto Rican Population

A. Rosado, I. Rivera, J.R. Paz, M.A. Noy

Dr. Noy Pathology Lab (Puerto rico)

Background / Objectives

A correlation exists between premalignant & malignant lesions and oncogenic Human Papilloma Virus (HPV) types. Segregation of HPV DNA has been accomplished from 46-100% of in situ & invasive Squamous Cell Carcinomas (SCCs) of the anus. Epidemiologic studies exhibit that up to 93% of anal SCCs are linked to HPV infection. The objective of this study is to compare the Anal High Risk (HR)–HPV infection between men & women within the Puerto Rican (PR) population, regardless of sexual orientation or lifestyle. Emphasized focus will prevail on HPV genotypes 16, 18 & 45, due to a shown tendency by PR medical professionals, to request analysis for these particular types.

Methods

337 anal Pap smear samples were collected from several medical clinics in PR from years 2013-2015. Samples were submitted through the following techniques: PCR analysis with Cobas® HPV Test & Aptima® HPV Assay, to identify HR-HPV genotypes; & through Flow Cytometry (FC) with HPV OncoProbe®, in order to detect the presence of viral activity.

Results

131 women were tested for HR-HPV: 4 were positive for HPV-16; 10 were positive for HPV-18: & 75 were positive for an unknown HR-HPV genotype. 206 men were tested for HR-HPV: 20 were positive for HPV-16; 9 were positive for HPV-18; & 38 were positive for an unknown HR-HPV genotype.

Conclusion

46% of the PR general population showed positive results for Anal HR-HPV infection. Distinctively, PR women seem to have a higher rate of Anal HR-HPV infection, with a 68% of female cases being positive for HPV-16, 18 or an unknown HR genotype. PR men are affected, but at a lesser rate of Anal HR-HPV infection, with a 33% of male cases being positive for HPV-16, 18

or an unknown HR genotype. Further analysis is necessary to identify the unknown HR genotypes, which are currently affecting the general PR population.

References

1] Colón-López, V. et al. (2014). Epidemiology of Anal HPV Infection in HR Men Attending a Sexually Transmitted Infection Clinic in PR. PLoS ONE, 9(1), e83209.
<http://doi.org/10.1371/journal.pone.0083209>.

P07-13

German population-based analysis of risk factors for prevalent high risk HPV infections

L. Wang¹, J. Haedicke², P. Martus¹, T. Iftner²

¹Institute of Clinical Epidemiology and Applied Biostatistics, University Hospital of Tuebingen (Germany), ²Medical Virology, Section Experimental Virology, University Hospital of Tuebingen (Germany)

Background / Objectives

To date there are no data available on the risk factors leading to a high risk human papillomavirus (HPV) infection in the German female population. Using a large German population-based study, we investigate the effects of a variety of both clinical and lifestyle risk factors such as smoking, age, sexual behavior, contraception, and sexually transmitted disease (STD) status on acquisition of HPV infection.

Methods

Women aged 30-60 years attending routine cervical screening were invited to participate in the study (n=10,040). Cervical specimens were collected and tested centrally by liquid based cytology (LBC), Aptima HPV test (AHPV) and Hybrid Capture 2 (HC2) using the high risk probe. Women were also invited to fill in questionnaires about their lifestyle. Questionnaire results of 9027 women are available and were analyzed using single, multiple, and step-wise logistic regression models, including possible interaction effects, to determine the relevant factors for HPV infection, the outcome variable. In addition, we also incorporated multiple imputation (using 20 imputation data-sets), assuming a missing at random (MAR) imputation scheme to complete our data when responses were not present. Missing data of risk factors ranged from 0% to 30%, all possible covariates were used for imputation models.

Results

Among the 9027 women in the analysis, 8526 (94.4%) women have normal cytology findings, and 501 (5.5%), have a cytological diagnosis of ASCUS or LSIL or higher. Increasing age, categorized by decades, was found to have an inverse effect on probability of a high risk HPV infection (OR: 0.652, p<0.000). Giving birth (dichotomized by previous birth or never having given birth) also decreased the risk of developing high risk HPV infection (OR: 0.58, p=0.001). While our survey data included the specific contraceptive method (including oral contraceptive (OC) use, condoms, IUD etc), we found

that the only relevant contraceptive factor was whether the women used OC, with OC usage increasing likelihood of infection (OR 1.64, $p < 0.000$). Smoking (dichotomous) also increases probability of infection (OR: 1.29, $p < 0.000$).

Conclusion

We found that certain lifestyle factors, primarily related to sexual behavior and smoking, individually have a strong association with high risk HPV infections. However, a significant interaction effect between different factors was not observed. Additionally, we confirmed previous European study findings that increasing age decreases risk of high risk HPV infections.

P07-14

EXAMINING LUNG CANCER TISSUE FOR HUMAN PAPILLOMAVIRUS

E. Argyri¹, E. Tsimplaki¹, C. Marketos², G. Politis², E. Panotopoulou¹

¹Virology Department, Saint Savvas General Anticancer Hospital, Athens, Greece (Greece), ²Pulmonary Department, Saint Savvas General Anticancer Hospital, Athens, Greece (Greece)

Background / Objectives

Lung cancer is the leading cancer worldwide among men and women with morbidity reaching 1.6 million. Human Papillomavirus is the causal factor of cervical cancer, as well as a subset of oropharyngeal cancers, while its association with others is still under investigation. The purpose of this study was to examine lung cancer tissues for the presence of HPV and its possible implication in lung oncogenesis.

Methods

Lung tissues were collected during bronchoscopy from 60 patients. One part of the tissue was referred for biopsy and the other part was subjected to HPV testing. Nucleic acids were extracted using the QIAamp DNA Mini Kit (Qiagen). PapilloCheck® HPV-Screening (Greiner Bio One) was used for the type-specific identification of 24 types of HPV (15 high-risk types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, probable high risk types 53, 66 and 7 low-risk types: 6, 11, 40, 42, 43, 44/55 and 70). A commercial real time NASBA assay (NucliSENS EasyQ HPV 1.1, bioMerieux) was performed for the qualitative detection of HPV E6/E7 mRNA of five high-risk HPV types (16, 18, 31, 33 and 45) according to the manufacturer's instructions.

Results

60 lung tissue samples were analysed. The age range was 49-85 years old (y.o) with a mean age of 67.7 y.o. 7 patients were female and 53 were male. All patients had cancer: the study included 9 Small Cell Lung Cancers (SCLC) and 51 Non Small Cell Lung Cancer (NSCLC) (28 AdenoCa, 20 SCC and 3 not defined NSCLC). 53 patients were smokers, 6 were former smokers and 1 was non smoker. Two patients were found positive in the HPV test: a male smoker with SCLC and a female smoker with AdenoCa. The two positive samples were subjected to E6/E7 mRNA test and were found negative. Furthermore, the two positive patients had no prior history of an HPV related disease.

Conclusion

Using the mRNA test as a gold standard for the association of HPV with malignant transformation, the present results showed no association of HPV status with lung cancer. Further investigation of more lung cancer tissues is required to reach safe conclusions.

P07-15

GENITAL WARTS IN SCANDINAVIAN WOMEN BEFORE AND AFTER IMPLEMENTATION OF HPV VACCINATION

C. Munk¹, M. Nygaard², L. Arnheim-Dahlström³, K.E. Juul¹, L.T. Thomsen¹, K.L. Liaw⁴, B.T. Hansen², S.K. Kjaer¹

¹Unit of Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen (Denmark), ²Department of Research, Cancer Registry of Norway, Oslo (Norway), ³Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden), ⁴Department of Epidemiology, Merck Research Laboratories, North Wales (United States of America)

Background / Objectives

A decreasing incidence of genital warts (GW) can be a first indicator of the effect of HPV vaccination if the qHPV vaccine, which includes HPV types 6 and 11, is used in the population under study. Based on two large cross-sectional surveys in Scandinavia performed before and after licensure of the qHPV vaccine, we examined the self-reported occurrence of GW as well as other sexually transmitted infections (STIs) to evaluate the impact of HPV vaccination with different implementation strategies between the three Scandinavian countries.

Methods

In 2004/5 (pre-vaccine) and in 2011/12 (post-vaccine), we conducted two questionnaire-based surveys in Scandinavia (Norway, Sweden and Denmark) among women 18-45 years of age (previously described in details^{1,2}). The questionnaires in both surveys included detailed information about sociodemographic and lifestyle variables, sexual behavior, HPV vaccination and STIs. We assessed the proportions of women reporting to have had GW and other STIs in the pre- and post-vaccination periods; overall and by selected age groups and country.

Results

In the first survey, we included 54,463 women (participation rate 71.3%) and in the second survey 48,788 women (60.6%). Overall, the proportion of women reporting a history of GW was 10.2% and 10.8% respectively in the first and second survey. Considering only the youngest women (18-19 years of age), the proportion in respectively Denmark, Sweden and Norway was 3.3%, 3.5%, 2.8% in the first survey and 1.9%, 3.3%, 2.3% in the second survey. The proportion of women

reporting *Chlamydia trachomatis* and gonorrhoea remained the same or increased both overall and in the youngest age groups between the two surveys.

Conclusion

In Denmark where high HPV vaccination coverage was achieved early through the childhood vaccination program, the incidence of GW decreased in the youngest age group targeted by the program. A similar decrease was not observed in Norway and Sweden that had different implementation strategies. The incidences of other STIs remained constant or increased in the meantime, indicating that sexual activity did not decrease between the two surveys.

References

1. Kjaer SK, Tran TN, Sparén P, Tryggvadóttir L, Munk C, Dasbach E, Liaw KL, Nygård J, Nygård M. **The burden of genital warts: a study of nearly 70 000 women from the general female population in the 4 Nordic countries.** *J Infect Dis* 2007; 196:1447–1454
2. Hansen BT, Kjær SK, Arnheim-Dahlström L, Liaw KL, Jensen KE, Thomsen LT, Munk C, Nygård M. **Human papillomavirus (HPV) vaccination and subsequent sexual behaviour: evidence from a large survey of Nordic women.** *Vaccine* 2014; 32:4945-53. doi: 10.1016/j.vaccine.2014.07.025.

P07-16

TRACE ELEMENTS AND OXIDATIVE STRESS IN CERVICAL CARCINOGENESIS

R. Zappacosta ¹, F. Di Toro ¹, A. Consalvo ², R. Lattanzio ², F. Sablone ¹, S. Rosini ¹, D. Ciavardelli ²

¹Department of medical, oral sciences and biotechnology (Italy), ²Center of Excellence on Aging (Ce.S.I.) (Italy)

Background / Objectives

Although recognised as essential to induce cervical cancer, infection by oncogenic HPV is not per se sufficient to lead to tumour development. Epidemiological evidences indicate that the vast majority of cervical lesions would regress spontaneously, while a small proportion of those expressing E6/E7 oncoproteins would have the potential to progress towards cancer. Since it is not possible to certainty predict the clinical outcome of a single HPV infection, all cervical lesions have to be regarded as potentially progressing. Thus, they often undergo overtreatment. The immunomodulatory role of trace elements as well as their influence on the outcome of many viral infection are widely recognized. Changes in serum levels of trace metals have been also observed in some neoplasia, thus postulating their role during carcinogenesis and cancer progression. Trace elements would also act as cofactors of antioxidant enzyme, which protects body from damage by oxidative stress (OS) that has a key role in several degenerative processes, including cancer. Based on this background, we firstly assessed the level of trace elements on a set of normal, dysplastic (E6/E7 mRNA positive) and neoplastic cervical samples. Further, we tentatively assessed the correlation with the expression of OS response proteins.

Methods

Trace elements were analysed on cytologic samples by Inductively Coupled Plasma Mass Spectrometry. On the corresponding tissue samples, expression pattern of OS associated proteins (CuZn Superoxide Dismutase 1/2-SOD1/SOD2, Thioredoxin Reductase2-TrxR2, Glutathione Transferase1-GSTP1) were investigated by immunohistochemistry. One-way ANOVA followed by Fisher LSD post-hoc test was performed to test for differences in elements concentration and proteins expression. p-values less than 0.05 were considered as statistically significant.

Results

Copper (Cu) level showed a trend to higher levels starting from negative to dysplastic groups. Then, it declined in neoplasia ($p < 0.001$). Zinc levels paralleled Cu concentrations, but this trend did not reach statistical significance. TrxR2 significantly increased its expression in dysplastic and neoplastic tissue, as compared with control. This trend was also observed for SOD1 and SOD2. GSTP1 shows an growing trend too, but its level fell in neoplastic tissue

Conclusion

Change in copper levels seems to be associated with increased oxidant milieu, which would provide the condition for neoplastic progression. Although further studies are needed, a better understanding of interdependence between stress protein markers and trace elements may provide further insights into the mechanisms involved in cervical carcinogenesis.

References

1. Jing Ji et al. Comparison of Serum and tissue levels of trace elements in different models of cervical cancer. *Biol Trace Elem Res* 2014; 159:346-50
2. Chaturvedi UC et al. Viral infections and trace elements: a complex interaction. *Curr Sci* 2004; 87(11):1536-54

P07-17

HPV-RELATED GENITAL DISEASE AMONG MEN RESIDING IN MEXICO

E. Lazcano-Ponce¹, M. Quiterio¹, S. Sudenga², A. Giuliano², J. Salmeron³

¹Instituto Nacional de Salud Publica (Mexico), ²H. Lee Moffitt Cancer Center (United States of America), ³Instituto Mexicano del seguro Social/ Instituto Nacional de Salud Publica (Mexico)

Background / Objectives

Despite the availability of primary prevention of HPV in men, Mexico has not yet introduced vaccination in the male population, focusing exclusively on girls 9-10 years old. The burden of condyloma (genital warts) in Mexico is not quantified; however, it may be as important as it is in other low- or middle-income countries. Assess the incidence of histopathologically confirmed condyloma and penile intraepithelial neoplasia (PeIN) and the HPV genotypes detected within these lesions.

Methods

HIM Study participants from Mexico were included in this country specific analyses. Participants were men aged 18-70 years living in Cuernavaca (central Mexico), enrolled between July 2005 and June 2009. Every six months beginning in 2009, participants underwent an interview, a physical exam, and laboratory analysis. Men who had two or more study visits after implementation of the protocol were included in this analysis (n=954). We collected a tissue sample from each external genital lesion (EGL) by shave excision. EGLs were categorized as condyloma, suggestive of condyloma, low- and high-grade squamous intraepithelial lesion (penile intraepithelial neoplasm, PeIN), or not HPV-related lesions. The Linear Array genotyping method was used to identify HPV genotypes from genital swabs, while the INNO-LiPA HPV Genotyping Extra method was used for biopsy tissue specimens. EGL incidence was computed among men free of EGL at baseline. Kaplan–Meier curves for EGL incidence were generated. Cumulative incidence of EGL within the first 12 months of follow-up was also estimated using Kaplan–Meier method.

Results

The EGL incidence rate (95% CI) was 1.84 (1.42-2.39) per 100 person-years; the cumulative risk of EGL at 12-months was 2.9% (1.9-4.2). The highest incidence of EGL was observed in the age group 18-30 years: 1.99 (1.22-3.25) per 100 person-years.

HPV-6 was the most common HPV genotype in condylomas (62.7%). Seven subjects developed PeIN 1-3, four of which contained HPV-16. Forty-four percent (14.3%-137.8%) of HPV 11 infections progressed to condyloma within 6-months after infection.

We found statistically significant differences in high-risk sexual behaviors between men that did developed an EGL compared to men that did not develop an EGL such as having sex with other men or having sex with both men and women were found, as well as total number of female and male partners.

Conclusion

HPV-related EGLs among men is very common in Mexico with 10% developing an EGL during follow-up. The burden of disease is similar in three geographical areas that have been studied (Cuernavaca, Tampa and Sao Paulo).

P08-01

The HPV 16 complete genome in two cervical progressive lesions from a 39-Year-Old Woman differ from each other.

R.H. Barbosa ¹, C.L. Machado ¹, F.N. Carestiato ¹, F.A. Silveira ², M.R.L. Passos ³, S.M.B. Cavalcanti ¹

¹1- Laboratório de Diagnóstico Viroológico, Departamento de Microbiologia e Parasitologia - Instituto Biomédico, UFF (Brazil), ²2- Instituto de Ginecologia – Universidade Federal do Rio de Janeiro. (Brazil), ³3- Setor de doenças sexualmente transmissíveis, Departamento de Microbiologia e Parasitologia - Instituto Biomédico, Universidade Federal Fluminense. (Brazil)

Background / Objectives

Background: Human papillomavirus (HPV) is a causal agent for approximately 5.3% of cancers worldwide and is the main cause of cervical cancer¹. HPV16, designed "high-risk" causes over half of all cervical cancer and some HPV16 variants are more oncogenic than others². Persistent high grade SIL squamous intraepithelial lesions (HSILs), represents the precursor lesion of cervical cancer³. It is still to be elucidated if included nucleotides vary across and within HPV types and lineages, or which of the single nucleotide polymorphisms (SNPs) determine oncogenicity². Objective: To characterize the HPV 16 genome of a rapidly progressive, cervical lesion in a 39-year-old woman.

Methods

Methods: Cervical biopsies from one woman with different HPV 16 lesions at two distinct time diagnosis (LSIL and progressive HSIL, with seven months interval) were analyzed by PCR and complete genome sequencing.

Results

Results: The most frequently observed variations were in E7, in L1, and in the LCR regions and novel variants were identified.

Conclusion

Conclusions: Our data suggest that specific SNPs are associated with increased risk for severe cervical lesion and neoplastic evolution. Molecular mechanisms involved in SNPs in HPV 16 and tumor initiation and progression require further investigation.

References

References:

1-Roden RB, Monie A, Wu TC. Opportunities to improve the prevention and treatment of cervical cancer. *Curr Mol Med*. 2007 Aug;7(5):490-503.

2-Smith B, Chen Z, Reimers L, Doorslaer K, Schiffman M, DeSalle R, Herrero R, Yu K, Wacholder S, Wang T, D. Burk RD. Sequence Imputation of HPV16 Genomes for Genetic Association Studies. *PLoS One*. 2011; 6(6): e21375.

3-Burd. EM Human Papillomavirus and Cervical Cancer. *Clin Microbiol Rev*. 2003 Jan; 16(1): 1–17.

P08-02

ANALYTICAL PERFORMANCE OF HPV GENOTYPING METHODS ON CERVICAL LIQUID BASED CYTOLOGY SAMPLES

M. Hansen ¹, H. Kristiansen-Haugland ¹, J. Šaltyte Benth ¹, E. Myrvang ¹, R. Meisal ¹, T. Rounge ², I.K. Christiansen ¹

¹Department of Microbiology and Infection Control, Akershus University Hospital, Lørenskog (Norway), ²Cancer Registry of Norway, Oslo (Norway)

Background / Objectives

The objective of this study is to evaluate the analytical performance of three human papillomavirus (HPV) genotyping methods on cervical liquid based cytology (LBC) samples. HPV genotyping is primarily important for epidemiological research and vaccine surveillance, but is increasingly also used for clinical purposes.

Methods

Consecutive SurePath LBC samples with diagnosis ASC-US or LSIL from routine screening are included in the study (n=200). HPV genotyping methods to be compared are: A method based on MGP-PCR followed by hybridization (Luminex technology) detecting and genotyping 37 HPV types; Anyplex HPV28 (Seegene) detecting and genotyping 28 types; and HPV next generation MGP amplicon sequencing using the Illumina MiSeq desktop sequencer. Cobas 4800 HPV test results (clinical test detecting 14 high-risk types) are available for the samples, allowing a relative comparison of the non-clinical genotyping test results to clinical test results.

Results

To date, all samples have been analysed with Luminex and Anyplex HPV28. In total, 168 samples (84%) were found positive for one or more HPV types; 32 samples (16%) were negative by both methods. A general observation is that the Anyplex assay has a tendency to detect more types than Luminex; up to eight types are reported in one sample. Altogether, 35 of the 38 genotypes covered by the two methods were identified. When comparing results for the 27 shared HPV types, a good overall agreement is observed. Considering the positive agreement, this is found low (<60%) for 9 of the 27 types. The negative agreement is good (well above 90%) for all types. Kappa values vary from 0.95 (0.90; 1.00) for HPV16 to 0.19 (0.003; 0.37) for HPV54. According to matrix correlation (RV) between Luminex and Anyplex, the measurements correlated moderately when considering all 27

types (RV=0.66), strongly among the 14 high-risk types (RV=0.79), and moderately among the remaining 13 types (RV=0.42). Of the 168 positive samples, 34 were negative with the Cobas test, of which 18 were identified with types other than the 14 included in the Cobas test.

Conclusion

For the two genotyping assays, a good correlation in terms of positive and negative samples is seen. Considering multiple infections, Anyplex HPV28 has a tendency to detect more types than the Luminex method. Both methods have a higher analytical sensitivity compared to the clinical Cobas 4800 HPV test. Correlations also including data from next generation amplicon sequencing will be presented.

P08-03

MicroRNA-106b promotes cell migration by targeting DAB2 in cervical carcinoma

L. Geng, Y. Cheng, J. Piao, Z. Li, Y. Zhang

Peking University Third Hospital (China)

Background / Objectives

Objectives: The role of miR-106b and its target gene DAB2 (disabled-2) on the migration of cervical cancer cells was explored.

Methods

The mRNA expression of miR-106b and DAB2 in cervical samples was detected using real time quantitative PCR. The protein expression of DAB2 was examined by Western blot. Dual luciferase reporter assay was used to identification of DAB2 as a miR-106b-directed target gene. Scratch and transwell assay were used to determine the effects of miR-106b and DAB2 on the migration of Hela cells.

Results

The expression level of miR-106b was clearly up-regulated in cervical cancer tissues. On the contrary, DAB2 expression was decreased in cervical cancer specimens. Dual luciferase reporter assay showed that the relative luciferase activity of WT-DAB2-3'UTR decreased approximately 30% after overexpression of miR-106b in HEK293T cells, the results of Mut-DAB2-3'UTR had no difference compared with the control group. DAB2 was identified as a miR-106b-directed target gene. Overexpression of miR-106b in Hela cells significantly promoted cell migration compared with the control group ($P < 0.05$). However, inhibition of DAB2 with siRNA, the rate of migration was increased remarkably ($P < 0.05$).

Conclusion

miR-106b promotes the migration of cervical cancer cells by directly targeting DAB2. These data suggested that miR-106b and DAB2 could play an important role in the pathogenesis of cervical carcinoma, and

miR-106b may be as a candidate of biomarker and a potential therapeutic target in cervical cancer.

P09-01

History and future of HPV College

T. Fait¹, V. Dvorak²

¹Dept. of Gynecologic and Obstetrics, General Faculty Hospital and 1st Faculty of Medicine, Charles University Prague (Czech republic), ²Centrum of outpatient gynecology and primary care, Brno (Czech republic)

Background / Objectives

To show daily work, history and future ideas of HPV College.

Methods

Observation study.

Results

HPV college is NGO. It has established in 2009 under patronage of Madeleine Albright . Members are gynecologists with expertise in HPV associated diseases. Foundation was motivated by lots of incorrect or incomplete information about HPV diseases prevention among both lay public and healthcare professionals. The aim of College is public education, healthcare professionals education, cooperation with media and interdisciplinary cooperation. Public education is based on web site with information about HPV problematic and anonymous chat with doctors. The most frequent questions among visitors of HPV College web will be presented.

Conclusion

Work of NGO in HPV could be important especially for lay public. We must to be strong opposition against antivaccination and antimedicinal movement.

References

P09-02

POPULATION-BASED STUDY ABOUT THE KNOWLEDGE OF WOMEN ON SECONDARY PREVENTION OF CERVICAL CANCER.

M. Hernández Aragón¹, C. Abad Rubio², B. Obón Castel¹, O. Lafalla Bernad¹, L. Ruiz Campo¹, J.M. Ramón Cajal¹

¹FEA Obstetrics and Gynecology Hospital San Jorge (Huesca) (Spain), ²MIR Obstetrics and Gynecology Hospital San Jorge (Huesca) (Spain)

Background / Objectives

We study the knowledge of feminine general population about the secondary prevention of cervical cancer.

Methods

We made a descriptive study of the level of knowledge about the screening of cervical pathology and its tracking in women between 25 and 65 years from population of Hospital San Jorge area (Huesca). We conduct 1028 surveys in different spheres, classifying women in four age groups: 25-34 years, 35-44, 45-54 and 55-65. The items to study includes: different techniques of screening, their objective, efficacy, frequency, reason of nonfulfillment, date of last cytology, possibility of self-sampling and origin of the information.

Results

From 1028 women who made the survey, 258 (25.1 %) were between 25-34 years old, 269 (26.2%) 35-44 years, 250 (24.3%) 45-54 years and 251 (24.4%) 55-65 years, with an average of 44 ± 11.6 years. 36.6% of women fulfil the period of 3 years between each cytology. 3.3% of women had never started the screening, and particularly the percentage increase to 7.4% in the youngest group age, finding significant differences with the rest of groups. 53.3% of women had get the last cytology less than a year ago. The principle reasons for not fulfil the screening was because they thought not needing it (49.9%), and the ignorance of the existence of a screening program (24.6%). 70.5% is in favour of a self-sampling program. 87.7% think cervical cancer is preventable with the screening program and 83% of women know that this screening can diagnose precancerous lesions. However, 28.8% of women do not know the exact objective of the screening program and 64.9% of women didn't know what other kind of cancers have a screening program. 33.7% received information from their gynaecologists, 4.6% from their midwives and 25.8% have no information.

Conclusion

Cytology is a widespread technique in population; however, there are differences in the tracking of protocol. The information about the objective of the screening and the importance in the fulfilment could improve with collaboration of professionals, as much from gynaecologists as from midwives.

P09-03

STUDY ABOUT THE ANXIETY GENERATED BY THE RESULT OF SCREENING TECHNIQUES OF CERVICAL CANCER

L. Ruiz Campo¹, C. Abad Rubio², O. Lafalla Bernad¹, B. Obón Castel¹, M. Hernández Aragón¹, J.M. Ramón Cajal¹

¹FEA Obstetrics and Gynecology Hospital San Jorge (Huesca) (Spain), ²MIR Obstetrics and Gynecology Hospital San Jorge (Huesca) (Spain)

Background / Objectives

We study the degree of anxiety associated to the result of screening techniques of cervical cancer depending on a positive or negative outcome.

Methods

We made a descriptive study about the degree of anxiety produced by the application of screening techniques of cervical cancer in women between 19 and 72 years from population of Hospital San Jorge area (Huesca). The anxiety is evaluated answering to a section from Goldberg Depression and Anxiety Scale before the medical test and after, depending on the outcome. (Anxiety \geq 4 points).

Results

The survey was made by 210 women, with a mean age of 40.5 ± 11.2 years. 92.4 % of screening results were negative and 7.6% were positive. 85.7% of women had less than 4 points in Anxiety Scale before the screening and after receiving the result, 90.4% had less than 4 points in the same scale. 18% of women with a positive result in the screening test have answered affirmatively to anxiety questions; meanwhile the percentage of these answers in women with negative outcome in the screening is 8.7%. There are significant differences regarding to irritability, difficulty to get relaxed and concern on their health.

Conclusion

The degree of anxiety produced by screening techniques is low. Without getting to achieve Anxiety standard, if we analyse one by one the questions, patients with a positive result of screening show a higher level of stress.

P10-01

COMPARATIVE EVALUATION OF THREE HPV GENOTYPING ASSAYS ON FFPE SAMPLES OF HEAD AND NECK CANCER

J. Mossong, M. Duvivier, J. Tapp, D. Stieber, F. Schmitt

Laboratoire National de Santé (Luxembourg)

Background / Objectives

HPV is a known risk factor for certain subgroups of HNSCC (head and neck squamous cell carcinoma) affecting disease prognosis and survival. HPV testing of FFPE (formalin fixed paraffin embedded) samples is becoming a routine in pathological practice, however there is no consensus on which methods to use for identifying HPV. In this study we compared 3 different methods to detect and genotype HPV DNA in archival FFPE samples of HNSCC.

Methods

Samples from 67 patients (mean age 62 years, 51 men, 16 women) diagnosed with HNSCC in 2014 were obtained from the service of pathology of the Laboratoire national de Santé in Luxembourg. DNA was extracted from FFPE cuts using Qiagen kits. HPV testing was conducted on all samples using the Cobas HPV assay (Roche, Switzerland). All positive and a subset of negative samples was also tested by EuroArray HPV (EuroImmune, Germany) and Anyplex II HPV28 (Seegene, Korea) assays according to manufacturers' instruction. For the Cobas assay, cycle threshold (CT) values indicative of viral concentrations were obtained using the open LC480 software. One sample with non-amplifiable positive control DNA in the Cobas assay was disregarded for further analysis.

Results

Based on the Cobas assay, 24 (36%) and one (1.5%) of the 66 samples were positive for HPV 16 or other high risk HPV (hrHPV), respectively. All 25 HPV positive samples and a randomly chosen subset of 19 negative samples were retested by Anyplex and EuroArray. Agreement (cf. Table) between Cobas and EuroArray was higher (kappa 0.78) than between Cobas and Anyplex (kappa 0.5). Anyplex detected 13 hrHPV and EuroArray 20 hrHPV samples. Both EuroArray and Anyplex detected genotype 66 for the other hrHPV sample detected by cobas. Samples positive by Anyplex and

Euroarray assays had significantly lower CT values in the Cobas assay than negative samples (Anyplex: 30.5 vs 39.9, $p < 0.0001$; EuroArray: 33.6 vs 41.1, $p = 0.01$).

Table: Concordance between EuroArray and Anyplex assays compared to Cobas assay

hrHPV genotype	+/+	-/-	+/-	-/+	Agreement	Kappa
Euroarray	20	19	0	5	88.6%	0.78 ($p < 0.0001$)
Anyplex	13	19	0	12	72.3%	0.5 ($p < 0.0001$)

Conclusion

Depending on the assay, 20-38% of HNSCC samples were found to be positive for hrHPV. Almost all (96%) of the HPV-positive HNSCC cancers were associated with HPV16. All three methods yielded concordant detection and typing results when viral loads were relatively high. Agreement between methods appears to be related to viral concentration. Although in our study the Cobas assay appears to be the most sensitive method for detecting hrHPV in FFPE samples, further work is needed to establish clinically relevant CT cut-offs.

P10-02

Prevalence of human papillomavirus infection in the oropharynx and urine among sexually active men: a comparative study of infection by papillomavirus and other organisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma* spp., and *Ureaplasma* spp

K. Nakashima¹, S. Kazuyoshi¹, K. Shohei¹, W. Akira², K. Yoshitomo³, N. Kazuyoshi⁴, S. Masayoshi⁵, S. Toshiyuki⁶, K. Yasuhide¹, M. Atsushi¹, N. Mikio¹

¹Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science (Japan), ²Wakatsuki Clinic (Japan), ³Department of Urology, Dokkyo Medical School, Koshigaya Hospital (Japan), ⁴Nakashima Clinic (Japan), ⁵Department of Urology, Nomi Hospital (Japan), ⁶Department of Obstetrics & Gynecology, Kanazawa Medical School (Japan)

Background / Objectives

Oropharyngeal squamous cell carcinoma (OSCC) has shown a gradual increase in male predominance due to the increasing incidence of human papillomavirus (HPV)-associated OSCC. However, the mode of HPV transmission to the oral cavity is poorly understood, and little is known about the epidemiology of oral HPV infection in men. The prevalence rates of HPV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma* spp., and *Ureaplasma* spp. were compared in the oropharynx (oral cavity) and urine of male Japanese patients attending a sexually transmitted disease clinic.

Methods

The study population consisted of 213 men aged 16 – 70 years old (mean: 34.4 years old). Oropharyngeal gargles and urine were collected, and sedimented cells were preserved in liquid-based cytology solution. After DNA extraction, β -globin and infectious organisms were analyzed by a PCR-based method. The HPV genotype was determined by HPV GenoArray test.

Results

β -Globin was positive in 100% and 97.7% of oral and urine samples, respectively. HPV detection rates were 18.8% and 22.1% in oral and urine samples, respectively, suggesting that the prevalence of HPV infection in the oral cavity was similar to that in the urinary tract. *N. gonorrhoeae* was more prevalent in oral (15.6%) than urine samples (9.1%), whereas *C. trachomatis* was detected more frequently in urine (15.9%) than oral samples (4.2%). The detection rates of *M. genitalium*, *M. hominis*, and *Ureaplasma* spp. were 5.2%, 10.3%, and 16.0% in oral samples, and 7.7%, 6.3%, and 19.2% in urine, respectively. There were no significant differences in the detection rates of *Mycoplasma* spp. and *Ureaplasma* spp. between anatomical locations. The distribution of HPV types were similar in oral and urine samples, and HPV16 was the most common type. The majority of men with HPV infection in both the oral cavity and urine had concordant oral and urinary HPV infection. The presence of urinary HPV infection was an independent risk factor of oral HPV infection, with an odds ratio of 3.39 (95% CI: 1.49 – 7.71), whereas oral gonococcal infection was inversely correlated with oral HPV infection (odds ratio: 0.096; 95% CI: 0.01 – 0.77).

Conclusion

In conclusion, oral HPV infection is common in sexually active men, and its prevalence rate was equivalent to that in urine samples. In addition, oral HPV infection was significantly correlated with urinary HPV infection.

References

1. Zur HH: Papillomavirus infections: a major cause of human cancers. *Biochem Biophys Acta* 1996, 1288:F55–78.
2. Miralles-Guri C, Bruni L, Cubilla AL, Castellsagué X, Bosch FX, de Sanjosé S: Human papillomavirus prevalence and type distribution in penile carcinoma. *J Clin Pathol* 2009, 62:870–878.
3. Hoots BE, Palefsky JM, Pimenta JM, Smith JS: Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer*

2009, 124:2375–2383.

4. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML: Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol* 2008, 26:612–619.
5. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, et al: Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011, 29:4294–4301.
6. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, et al: Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010, 363:24–35.
7. Tanaka S, Sobue T: Comparison of oral and pharyngeal cancer mortality in five countries: France, Italy, Japan, UK and USA from the WHO Mortality Database (1960-2000). *Jpn J Clin Oncol* 2005, 35:488–491.
8. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, et al: Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000, 92:709–720.
9. Smith JS, Melendy A, Melendy A, Rana RK, Pimenta JM: Age-specific prevalence of infection with human papillomavirus in females: a global review. *J Adolesc Health* 2008, 43(Suppl):S5–25.
10. Yamada R, Sasagawa T, Kirumbi LW, Kingoro A, Karanja DK, Kiptoo M, Nakitare GW, Ichimura H, Inoue M: Human papillomavirus infection and cervical abnormalities in Nairobi, Kenya, an area with a high prevalence of human immunodeficiency virus infection. *J Med Virol* 2008, 80:847–855.
11. Shigehara K, Sasagawa T, Kawaguchi S, Kobori Y, Nakashima T, Shimamura M, Taya T, Furubayashi K, Namiki M: Prevalence of human papillomavirus infection in the urinary tract of men with urethritis. *Int J Urol* 2010, 17:563–568.
12. Grisar D, Avidor B, Niv J, Marmor S, Almog B, Leibowitz C, Graidy M, Giladi M: Pilot study of prevalence of high-risk human papillomavirus genotypes in Israeli Jewish women referred for colposcopic examination. *J Clin Microbiol* 2008, 46:1602–1605.
13. Chaudhry U, Saluja D: Detection of *Neisseria gonorrhoeae* by PCR using *orf1* gene as target. *Sex Transm Infect* 2002, 78:72–78.
14. Mahony JB, Song X, Chong S, Faught M, Salonga T, Kapala J: Evaluation of the NucliSens Basic Kit for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in genital tract specimens using nucleic acid sequence-based amplification of 16S rRNA. *J Clin Microbiol* 2001, 39:1429–1435.
15. Shigehara K, Kawaguchi S, Sasagawa T, Furubayashi K, Shimamura M, Maeda Y, Konaka H, Mizokami A, Koh E, Namiki M: Prevalence of genital *Mycoplasma*, *Ureaplasma*, *Gardnerella*, and human papillomavirus in Japanese men with

urethritis, and risk factors for detection of urethral human papillomavirus infection. *J Infect Chemother* 2011, 17:487–492.

16. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA: Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol* 2004, 42:1528–1533.

17. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML: Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007, 356:1944–1956.

18. D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML: Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis* 2009, 199:1263–1269.

19. Kreimer AR, Villa A, Nyitray AG, Abrahamsen M, Papenfuss M, Smith D, Hildesheim A, Villa LL, Lazcano-Ponce E, Giuliano AR: The epidemiology of oral HPV infection among a multinational sample of healthy men. *Cancer Epidemiol Biomarkers Prev* 2011, 20:172–182.

20. Kreimer AR, Bhatia RK, Messegue AL, González P, Herrero R, Giuliano AR: Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis* 2010, 37:386–391.

21. Marais DJ, Passmore JA, Denny L, Sampson C, Allan BR, Williamson AL: Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *J Med Virol* 2008, 80:953–959.

22. Smith EM, Ritchie JM, Yankowitz J, Wang D, Turek LP, Haugen TH: HPV prevalence and concordance in the cervix and oral cavity of pregnant women. *Infect Dis Obstet Gynecol* 2004, 12:45–56.

23. Fakhry C, D'souza G, Sugar E, Weber K, Goshu E, Minkoff H, Wright R, Seaberg E, Gillison M: Relationship between prevalent oral and cervical human papillomavirus infections in human immunodeficiency virus positive and –negative women. *J Clin Microbiol* 2006, 44:4479–4485.

24. Edelstein ZR, Schwartz SM, Hawes S, Hughes JP, Feng Q, Stern ME, O'Reilly S, Lee SK, Fu Xi L, Koutsky LA: Rates and determinants of oral human papillomavirus infection in young men. *Sex Transm Dis* 2012, 39:860–867.

25. Kawaguchi S, Shigehara K, Sasagawa T, Shimamura M, Nakashima T, Sugimoto K, Nakashima K, Furubayashi K, Namiki M: Liquid-based urine cytology as a tool for detection of human papillomavirus, *Mycoplasma* spp., and *Ureaplasma* spp. in men. *J Clin Microbiol* 2012, 50:401–406.

26. Sasagawa T, Basha W, Yamazaki H, Inoue M: High-risk and multiple human papillomavirus infections associated with cervical abnormalities in Japanese women. *Cancer Epidemiol Biomarkers Prev* 2001, 10:45–52.

27. Takahashi S, Takeyama K, Miyamoto S, Ichihara K, Maeda T, Kunishima Y, Matsukawa M, Tsukamoto T: Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. *J Infect Chemother* 2006, 12:269–271.

28. Sackel SG, Alpert S, Fiumara NJ, Donner A, Laughlin CA, McCormack WM: Oro-genital contact and the isolation of *Neisseria gonorrhoeae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* from the pharynx. *Sex Transm Dis* 1979, 6:64–68.
29. Peters RP, Nijsten N, Mutsaers J, Jansen CL, Morr  SA, van Leeuwen AP: Screening of oropharynx and anorectum increases prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in female STD clinic visitors. *Sex Transm Dis* 2011, 38:783–787.
30. Giuliano AR, Nielson CM, Flores R, Dunne EF, Abrahamsen M, Papenfuss MR, Markowitz LE, Smith D, Harris RB: The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. *J Infect Dis* 2007, 196:1146–1152.

P10-03

PARP INHIBITION (OLAPARIB) IN NOVEL IN-VITRO MODELS OF OROPHARYNGEAL CANCER

E. Pirotte¹, P. Giles², K. Ashelford², A. Fiander¹, A. Al-Hussaini³, D. Owens³, M. Evans⁴, N. Powell¹

¹HPV Research Group, Cardiff University, School of Medicine, Cardiff (United kingdom), ²Institute of Medical genetics, Cardiff University, School of Medicine, Cardiff (United kingdom), ³Head and Neck surgery, University hospital of Wales, Cardiff (United kingdom), ⁴Clinical oncology, Velindre cancer center, Cardiff (United kingdom)

Background / Objectives

HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) is increasing in incidence in many developed countries. HPV infection appears to be associated with defects in DNA double strand break repair. Targeting these defects could facilitate less toxic treatment of OPSCC. The PARP-inhibitor Olaparib inhibits DNA base excision repair and can induce synthetic lethality in cells with deficient repair of double strand DNA breaks. Development of novel therapies for HPV-positive OPSCC is hampered by a lack of in vitro models. The aims of this project were to develop and characterise novel HPV-positive OPSCC cell lines, and use them to evaluate the potential of synthetic lethal therapies.

Methods

Novel cell lines were derived from OPSCC biopsies and characterised for HPV infection and integration, p53 status and STR profile. mRNA-seq analysis was used to compare gene expression (human and HPV) in the novel lines with a panel of established head and neck cancer cell-lines. Response to Olaparib was assessed in eight OPSCC cell lines using clonogenic assays, and flow cytometry (gamma-H2AX and cell cycle).

Results

Two novel cell lines derived from tonsil tumours in non-smoking men, showed the presence of HPV16 DNA, wild-type p53 and viral integration. Differential gene expression analysis showed that the novel lines (CU-OP-2 and CU-OP-3) shared a similar pattern of gene expression, which differed from that observed in two widely used HPV-positive lines (UMSCC-47 and UPCI-SCC-090), as well as from 4 HPV-negative OPSCC cell-lines (UMSCC-4, UMSCC-6, UMSCC-19 and UMSCC-74A). Olaparib treatment reduced colony formation in a dose dependent manner. Two HPV-positive cell lines were

sensitive to therapeutically relevant doses of Olaparib. Treatment with Olaparib caused an increase in double strand DNA breaks and accumulation in G2 phase.

Conclusion

Two novel OPSCC cell-lines have been generated and characterised. Some HPV-positive lines appear sensitive to Olaparib at potentially therapeutic doses. This suggests PARP inhibition may be useful for treatment of HPV-positive OPSCC.

P11-01

Effectiveness of HPV vaccine for the prevention of cervical abnormalities in Saitama City, Japan in 2015

D. Hotta¹, K. Chikazawa¹, Y. Akiyama², R. Konno¹

¹ichi Medical University Saitama Medical Center (Japan), ²Akiyama Ladies Clinic (Japan)

Background / Objectives

Objective

Human papillomavirus (HPV) vaccine was licensed in Japan in 2009. It was funded by a budget from both the national and local governments from 2011. The HPV vaccine program targets 12-16-year-old girls. The aim of this study was to evaluate the effectiveness of the vaccine by comparing trends in incidence rates of cytological abnormalities from cervical screening data in Saitama city, Japan.

Methods

Methods

Omiya Medical Association in Saitama city performs cervical cancer screening using cytology and ASC-US triage with HPV testing. Participants attending the screening program between April 2015 and January 2016 were enrolled in this study. At screening, women had to fill in a questionnaire about HPV vaccine status. Incidence of cytological abnormalities was compared between the group and unvaccinated group. ASC-US or worse was defined as a cytological abnormality.

Results

RESULTS

Out of all of 11,703 participants, 201 women (1.7%) were vaccinated and 11,502 women were unvaccinated. Overall cervical screening results showed that 5 cases (2.5%) with cytological abnormalities were in vaccinated women and 263 cases (2.3%) in unvaccinated women. In women aged 20-25yrs (birth years 1995-1990), coverage of HPV vaccine was 68.0%, 59.4%, 3.8%, 5.7%, 4.8% and 3.9%, respectively. The first and second vaccinated birth cohorts had relatively high coverage. Furthermore, the results of cervical screening from a group of 485 women of 20 to 25 years showed

2 cases (2.7%) in vaccinated women and 21 cases (5.1%) in unvaccinated women, respectively. Incidence risk ratio was 0.524 [95%CI : 0.120–2.286].

Conclusion

Conclusion

Incidence of cervical abnormalities seems to be decreasing in the cohort of young women with higher vaccine coverage, but as of 2015, it is not yet statistically significant. Further surveillance is necessary to evaluate the effectiveness of the HPV vaccine in Japan.

P11-02

THE POTENTIAL IMPACT OF PAPILOMAVIRUS VACCINES IN OPERABLE CERVICAL TUMOURS

M. Mouraz¹, A. Coelho², M. Rocha², M. Bernardino², M. Cunha³, A.F. Jorge²

¹Obstetrics and Gynecology Dept., Hospital Dr. José de Almeida, Cascais (Portugal), ²Gynecology Dept., Instituto Português de Oncologia Dr Francisco Gentil, Lisboa (Portugal), ³Clinical Pathology Dept., Instituto Português de Oncologia Dr Francisco Gentil, Lisboa (Portugal)

Background / Objectives

High-risk Human Papilloma Virus (HPV) is a major risk factor for cervical dysplasia and cancer. The current quadrivalent vaccine introduced in the National Vaccination Programme, with a coverage rate of 85%, will provide only some protection against cervical cancer, when it is linked to HPV 16/18. The objective of this study was to assess the potential impact of an HPV nonavalent vaccine in diminishing the cervical cancer burden.

Methods

An observational, retrospective, cross-sectional analysis was conducted to evaluate the distribution of high risk HPV genotypes among women with a cervical cancer who were referred and submitted to surgery in the Gynecological Department of Lisbon's Oncology Institute, from January 2012 to December 2015.

Results

A hundred and ten patients (mean age $49,45 \pm 10,95$ years) were included. Ninety two were HPV positive (83.6%). The majority of these tested positive for HPV 16 (67.4%) followed by HPV 18 (9.8%). A single HPV infection was present in 72.8%. The five most frequent types were HPV 16 (67.4%), HPV 18 (9.8%), HPV 33, HPV 53 and HPV 58 (6.5%). Multiple HPV types were present in 27.2%, with two HPV types in 17.4%, three HPV types in 7.6% and four or more HPV types in 2.2%. HPV type prevalence was 97.1%, 98.5%, 96.6% and 96.3% among low and high squamous intraepithelial neoplasia (LSIL and HSIL), adenocarcinoma (AC) and squamous cell carcinoma (SCC) cases, respectively. The most common HPV type in HSIL were HPV 16/53/33 (53.5/9.3/4.7%) and in invasive cervical cancer (ICC) were HPV 16/18/58 (53.7/13.0/5.6%). In SCC the more frequent HPV

identified were HPV 16/33/52 (66.9/5.7/5.7%) and in AC HPV 16/18/31 (46.7/13.3/6.7%). The positive rates of the HPV high-risk types (HPV 16 and 18) included in current prophylactic vaccines, represented 65.4% of women with SCC, 70.0% with AC, 100% with other type of ICC, as well as 53.6% with HSIL and 28.6% of LSIL cases. The nonavalent vaccine that includes 5 more HPV types (31/33/45/52/58) will cover 84.6% of the SCC, the same percentage of AC, 67.9% of HSIL and 57.1% of LSIL cases in our sample.

Conclusion

This study showed that in our population the nonavalent vaccine would potentially prevent more 7.7% of SCC. It might also potentially reduce the rate of HSIL in 14.3% and LSIL in 28.5%. Ideally if HPV 53 could be included, this reduction could be potentialized: more 11.5% of SCC cases, representing a coverage of 92.3%, and more 10.7% of HSIL, representing a coverage of 78.6%.

National registry is needed in order to follow the trends of specific HPV types in portuguese society and to evaluate the potential impact of profilatic measures, especially the use of HPV vaccines.

P11-03

HPV Vaccine Uptake Rate in 9-21 Year Old Adolescents during Well-Child Visits from 2007 to 2013 in the U.S.

D. Zhang, A. Kulkarni, B. Lindsay

Center for Observational and Real-World Evidence, Merck & Co., Inc., Kenilworth, NJ (United States of America)

Background / Objectives

The Advisory Committee on Immunization Practices (ACIP) recommends HPV vaccination for females aged 13-26 years and males aged 13-21 years not vaccinated previously, and routine HPV vaccination for 11 or 12 year olds. Well-child visits present the best opportunities for children and adolescents to receive vaccines. The objectives of this study were to estimate HPV vaccine uptake rate in females and males aged 9-21 years during well-child visits from 2007 to 2013 and to compare HPV vaccine uptake in adolescents aged 11-12 years old with Tdap and meningococcal vaccines.

Methods

This retrospective database study included 9-21 year old females and males who were continuously enrolled since June 1, 2006 or January 1 of the year when subjects turned to 9 years old, had well-child visits, didn't have HPV vaccine previously, were not pregnant, did not have a claim related to child birth, cervical cancer, or hysterectomy during the study year. Subjects were assessed for receipt of HPV vaccine during well child care visits. HPV vaccination was identified by CPT4 codes: 90649 and 90650. Subjects who initiated HPV vaccination during well-child care visits were followed for two years to assess 3-dose series completion. HPV vaccine uptake rates and completion rates were reported for the overall study cohort and stratified by age groups, gender, region, health plan type, and provider specialty type. Multivariable logistic regression models were used to analyze factors associated with HPV vaccine uptake and series completion.

Results

Across all years, well-child visit rate was highest among 11-12 year olds (40.1%-58.2%). HPV vaccine uptake rate during well-child visits was highest among adolescents 13-17 years of age (21.5%). Only 18.6% of adolescents between 11 and 12 years received the HPV vaccine. HPV vaccination rates in 11-12 years olds during well-child visits (18.6%) were significantly lower than Tdap (35.5%, $p < 0.0001$) and MCV4 (34.5%, $p < 0.0001$). Compared to other age groups, the 11-12 year olds were most likely to

come in for a well-child visit. The 13-17 year olds were most likely to get a HPV vaccine during the well-child visit (OR=1.207; p <0.001).

Conclusion

Though 11-12 year boys and girls were more likely to come in for a well-child visit, they were less likely to receive a HPV vaccine. Well child visits for 11 and 12 years olds are missed opportunities for HPV vaccinations.

References

Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007; 56:1–24.

Markowitz LE, Dunne EF, Saraiya M, Chesson HW, Curtis CR, Gee J, Bocchini JA Jr, Unger ER; Centers for Disease Control and Prevention (CDC). Human papillomavirus vaccination: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2014 Aug 29;63(RR-05):1-30.

American Academy of Pediatrics. Well-Child Care: A Check-Up for Success. <http://www.healthychildren.org/English/family-life/health-management/Pages/Well-Child-Care-A-Check-Up-for-Success.aspx>. Accessed Aug 19, 2014.

P11-04

9-VALENT HPV VACCINE DEVELOPMENT: INNOVATIVE REGULATORY APPROACH USING A PHASE IIB/III DESIGN AND AN IMMUNOLOGICAL BRIDGING TO ESTABLISH EFFICACY

A. Fisher ¹, F. Meunier ², A. Luxembourg ¹

¹Merck & Co., Inc., Kenilworth, NJ, USA (United States of America), ²Sanofi Pasteur MSD, Lyon, France (France)

Background / Objectives

The 9-valent HPV vaccine has the potential to prevent ~90% cervical cancers. An innovative regulatory approach was taken to accelerate development.

Methods

(1)An adaptive, seamless phase IIB/III design was used to proceed from dose selection (phase IIB) to phase III without pause to shorten the development time

(2)A hybrid approach was taken to define the primary endpoints.

i.An active comparator (Gardasil) had to be used.

ii.Since HPV vaccines are highly efficacious, few HPV6/11/16/18-related disease endpoints were expected, precluding a comparison based on efficacy.

iii.Efficacy for HPV6/11/16/18 was inferred based on non-inferior immunogenicity given the absence of immune correlate of protection

iv.Efficacy for the new HPV types was established based on efficacy endpoints

v.A hybrid approach for the primary endpoints facilitated the implementation of the seamless design.

Adaptive design with hybrid approach for primary endpoints is novel for vaccine development.

Careful planning and rigorous execution of the Phase IIB/III adaptive design and dialogue with regulators was critical to success.

Conclusion

The 9-valent HPV vaccine was licensed as Gardasil 9 in the US in Dec 2014, Canada in Feb 2015 and EU and Australia in Jun 2015. A case study of this approach is presented.

This regulatory approach shortened development time, reduced the number of subjects in trials and offers an innovative approach to vaccine development.

P11-05

NO INCREASE IN GUILLAIN-BARRE SYNDROME HOSPITALISATIONS AFTER HPV VACCINE PROGRAM IMPLEMENTATION: AN ADMINISTRATIVE DATABASE ANALYSIS IN QUÉBEC, CANADA

G. Deceuninck¹, C. Sauvageau², V. Gilca², N. Boulianne², G. De Serres²

¹Centre de recherche du CHU de Québec (Canada), ²Institut national de santé publique du Québec (Canada)

Background / Objectives

A publicly-funded school-based human papilloma virus vaccination (HPVV) program was implemented in the province of Québec, in 2008 (total birth cohort ≈85 000). Grade 4 girls (9-10 y-o) were eligible for routine vaccination. A catch-up vaccination was also in place for grade 9 girls (14-15-y-o) from 2008 to 2013. In both grades, annual vaccination coverage was 76-81%. A French document (Alpérovitch, 2015) has recently reported an association between HPV vaccination and Guillain-Barré Syndrome (GBS). The objective of this analysis was to assess the hospitalisations rates for GBS in HPVV targeted cohorts compared to non-vaccinated cohorts of the similar age, in the province of Québec.

Methods

Hospital discharge records of children aged 7 to 17 with a main diagnosis of GBS were analyzed. Age- and sex-specific incidence rates according to HPVV program eligibility were computed for the period October 1999 to March 2014. Sex, age, year of diagnosis, and H1N1 pandemic period were tested in multivariate analyses and adjusted relative risk in targeted cohorts was estimated by Poisson regression.

Results

One hundred SGB cases were retrieved and included in the analysis. The total hospitalisation rate for GBS in 7-17 year-olds was 0.73/100 000 person-years. Increasing age and H1N1 pandemic period were significantly associated with higher risk of hospitalisation for GBS. The adjusted relative risk of GBS in the HPVV targeted population (grade 4 and 9 girls) was estimated at 0.86 (95%CI: 0.29-2.26).

Conclusion

In Québec, no increase in hospitalisation rates for GBS was observed in HPVV targeted compared to non-targeted cohorts.

References

Alpérovitch A, Elbaz A, Faye A, et al. Vaccins anti-HPV et risque de maladies autoimmunes : étude pharmacoépidémiologique. Paris: Agence nationale de sécurité du médicament et des produits de santé, 2015, 91 p.

P11-06

STUDY OF HPV VACCINATION IN GREECE DURING THE LAST FOUR YEARS

A.N.A.S.T.A.S.I.A. Vatopoulou, T. Agorastos, T. Theodoridis, L. Athanasiadis

ARISTOTLES UNIVERSITY OF THESSALONIKI, THESSALONIKI, Greece (Greece)

Background / Objectives

The main aim is to present the level of awareness of the HPV virus, the acceptance of vaccination and the investigation of the reasons why there is still a percentage that refuses to be vaccinated. Also to present the main sources of information on the HPV and the figure, that played a decisive role on the acceptance of the vaccine.

Methods

A questionnaire consisting of 84 questions was filled by 300 young females between the ages of 12-25 years. The young women visited a Pediatric & Adolescent Gynecology clinic of a university hospital. These questionnaires were distributed and filled from January 2012 until January 2016. The questions were asked in a way to investigate the level of knowledge and awareness concerning the HPV, the related cancers, also about the HPV vaccine and whether these woman wanted to be vaccinated or not.

Results

By this study an improvement to the general acceptance of the vaccination was monitored, since during the first two years the 68,1% of the females asked were skeptical about receiving the vaccination and refused to get vaccinated, whereas during the second two years the percentage reduced to 41,7%. The main reasons of refusal with a percentage of 51,9% was the worry of the side-effects that may arise. What is however really important to mention was that 29,6% declared that nobody had suggested the vaccination or even worse that their family doctor was objecting to the vaccine.

Conclusion

There is an increasing knowledge on the vaccine and HPV virus stimulated by NHS. Still there is plenty of room for improvement, beginning with a more extensive information campaign by health

providers.

P11-07

The impact of the vaccination with bivalent HPV vaccine after electrosurgical conization in preventing of HPV infection in patients with high grade cervical intraepithelial neoplasia.

N. Volodko¹, V. Soboljeva , O. Petronchak . R. Huley, I. Palyha ²

¹Danylo Halytsky National Medical University in Lviv, Lviv (Ukraine), ²Clinic for Human Reproduction "Alrernatyva", Lviv , (Ukraine)

Background / Objectives

Persistent high-risk human papillomavirus (HR-HPV) is strongly associated with the development of CIN2–3, which is considered the the beginning of the progression to cervical cancer . Excision procedures (LLETZ, conization) are both a diagnostic and therapeutic procedures that can effectively eradicate CIN2–3 . However, , residual/recurrent disease associated with HR-HPV persistence after conisation varies between 5%- 30%, requiring retreatment of the lesions .

The aim of the study was to determine whether vaccination with the bivalent human papillomavirus (HPV) vaccine Cervarix after electrosurgical conisation for high-grade cervical intraepithelial neoplasia (CIN2 -3) is effective in preventing of HG-HPV persistence .

Methods

115 patients (mean age 35.5 years, range 23–47 years) with histologically verified CIN 2 or CIN 3 who underwent electroknife conization with negative margins between December 2011 and January 2014 were enrolled in this study.

55 patients were vaccinated with Cervarix after surgical treatment (vaccination group), and 60 patients were followed without vaccination (non-vaccination group). The patients in vaccination group received the first dose at 3 weeks after conisation and the remaining two doses one and six months later. Postconisation follow-up was performed at 3, 6, 9, 12, 18, and 24 months during the first 2 years and yearly thereafter. The cytology, colposcopy and HPV genotyping by PCR before surgery and at 8-12 months follow up was performed to all patients.

Results

High-risk HPVs were detected in the primary cervical lesions of 113 of 115 patients (97.1%) prior to conization. Follow-up at 12-16 months revealed that HR- HPVs were eradicated by conization in 80 % without vaccination. HPV-genotyping after treatment identified persistent viral infection in 12 of 60

(20%) nonvaccinated patients. In vaccinating group the presence of HG-HPV at the 12-14 month visits was found in 5 of 55 of patients (12.5 %). Persistence of HPV 51, 58 was revealed in 4 patients in vaccinated group. Persistence of HPV16 was found in one patient.

Conclusion

Vaccination with with the bivalent human papillomavirus (HPV) vaccine Cervarix after electroconization improved the level of HG-HPV elimination and may be considered in preventing recurrence of CIN2 –3.

P11-08

EARLY RESULTS OF HPV-VACCINATION PROGRAM WITH QUADRIVALENT HPV VACCINE DEPENDING ON VACCINE COVERAGE IN 3 DISTRICTS OF MOSCOW REGION, RUSSIA

V. Krasnopolskiy, N. Zarochentseva, J. Belaya, L. Keschjan, E. Bulycheva, L. Dzhidzhikhia, L. Kavteladze

The State budgetary Institution of obstetrics and gynecology of Moscow Region (Russian federation)

Background / Objectives

Moscow Region has been the first region in the Russian Federation that introduced HPV vaccination program for girls 13-15 years of age in 9 districts in 2008. Genital warts (GW) are the first clinical endpoint to study when investigating effectiveness of quadrivalent HPV vaccine. The aim of our study was to evaluate effectiveness of HPV vaccination program after 5 years of introduction in 3 districts of Moscow region with different coverage of vaccine.

Methods

Ecological study based on annual reports of gynecologists of adolescents on incidence of GW among 10-17 aged girls in 3 districts of Moscow region: Naro-Fominsk, Kolomna and Egorievsk with 4-valent HPV vaccine coverage in 13-15 aged girls population 80%, 30% and 0% respectively. Trends in incidence of GW were calculated from 2009 to 2014 among 10-17 aged girls.

Results

Significant decline in incidence of GW was observed in Naro-Fominsk district with 4-valent HPV vaccine uptake 80% (from 9,3 per 100 000 to 3,8 per 100 000), followed by Kolomna with 30% vaccination coverage (from 6,2 per 100 000 to 4,8 per 100 000). In contrast with Naro-Fominsk and Kolomna the incidence of GW in Egorievsk district (without introduced HPV vaccination program) had increased (from 8,4 per 100 000 to 10,1 per 100 000)

Conclusion

This data suggests that high-coverage HPV-vaccination programs may result in a rapid reduction of GW (first marker of effectiveness for 4-valent HPV vaccine). In the longer term, substantial reductions in the rates of cervical, vulvar, vaginal and anal cancers may follow.

P11-09

PUBLIC HEALTH IMPACT AND COST EFFECTIVENESS OF A UNIVERSAL VACCINATION PROGRAMME WITH A NONVALENT HPV VACCINE IN GERMANY

M. Uhart¹, J.A. Jacob², F. Bianic³, D. Anger³, N. LARGERON¹

¹SPMSD (France), ²MAPI (United kingdom), ³MAPI (France)

Background / Objectives

The nonavalent vaccine, by protecting against five additional oncogenic HPV types and nine HPV types in total (6, 11, 16, 18, 31, 33, 45, 52 and 58), is expected to prevent an even broader spectrum of HPV-related cancers and other diseases - 90% of cervical and 90% of HPV related anal cancers could be averted thanks to this new vaccine. The present study aims to estimate the incremental public health impact and cost-effectiveness of a universal vaccination programme with a nonavalent vaccine in Germany as compared to the current girls only quadrivalent (6/11/16/18) HPV vaccination

Methods

A dynamic transmission model including a wide range of health and cost outcomes related to cervical, anal, vulvar, vaginal diseases and genital warts was calibrated to German epidemiological data. The clinical impact due to the 5 new types was included for cervical and anal diseases only. In the base case, a two dose vaccination program with lifelong protection and a cumulative vaccination coverage rate of 55.6% was assumed in the cohorts aged 17. German costs, including official price for the vaccines, were used. Deterministic sensitivity analyses on key parameters (such as duration of protection, discount rate and inclusion of head and neck cancers) were conducted.

Results

It was estimated that after 100 years, the universal HPV9 vaccination programme would further decrease the vaccine type incidence of cervical cancer and precancers from 57% to 80%, of anal cancer from 64% to 78% in females and from 46% to 75% in males and of genital warts from 40% to 46% in females and from 16% to 38% in males. Overall, the new intervention would avert 46.454 additional cases of cervical cancers, 896.242 cases of precancers, 8.456 cases of anal cancers and 1.448.735 cases of genital warts compared to the current vaccination program. This new intervention would be cost-effective with an incremental cost per QALY of 22,990 €. Sensitivity analyses showed the ICER was significantly improved when all HPV diseases (RRP, penile and head and neck cancers) are included in the analysis.

Conclusion

The switch to a universal vaccination programme with a nonavalent vaccine in Germany is estimated to be cost-effective across a range of sensitivity analyses. Considering a coverage rate of 55%, the intervention could lead to a public health impact comparable to the one observed in other EU countries with a higher VCR in girls (70%).

P11-10

OVERCOMING BARRIERS TO ADOLESCENT VACCINATION: VACCINE CHAMPIONS' PERSPECTIVES

B. Sanusi¹, J. Smith², B. Meadows³, J.S. Myers³

¹University of North Carolina, Chapel Hill, North Carolina, USA (United States of America), ²UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina, USA (United States of America), ³North Carolina Immunization Branch, North Carolina Department of Health and Human Services, Raleigh, North Carolina (United States of America)

Background / Objectives

Coverage of recommended adolescent vaccines in the United States (US) lags notably behind childhood vaccination. We aimed to determine provider-associated factors which hinder adolescent vaccination as well as successful strategies to maximize adolescent vaccine uptake.

Methods

A total of 20 vaccine champions were recruited across counties in North Carolina (NC). Adolescent providers were identified with above average vaccine coverage rates for adolescents aged 13 through 17 within NC Vaccine For Children (VFC) clinical practices. Vaccination champions were interviewed using a semi-structured questionnaire to capture lessons learned for reducing missed opportunities for adolescent vaccination.

Results

Facilitators to adolescent vaccine program success included having systems to identify patients eligible for their first dose of HPV vaccine (95%); routinely providing vaccines to adolescents at well-child visits (95%) and acute visits (75%). Additional facilitators included providing training on adolescent immunizations to other clinic staff (85%); reminders for patients' vaccination due dates (80%); having standing orders for providing adolescent vaccines (75%); and involvement of NC Immunization Branch in vaccine uptake strategies with clinics (60%). Barriers faced in immunizing adolescents included parents' opposition due to lack of education or negative media (35%); HPV vaccine stock outs (35%); issues related to child's sexual activity (30%); concerns about pain following vaccination (15%); and dose completion problems (15%). Steps taken if parents refused or

wished to delay vaccines included trying again during next visit (45%); providing education materials (40%), discussing vaccine safety (20%); and asking questions to explore parents' concerns (15%). Treating HPV vaccine the same as required vaccines when recommending to parents and adolescents was cited as an opportunity for increased rate of HPV vaccination (95%).

Conclusion

Adapting these lessons learned to inform state-specific adolescent vaccination action plans could improve national HPV and other adolescent vaccination rates, and may be applicable to other global populations.

P12-01

HPV infection among HIV-positive men: A three year revised experience of an diagnosis Laboratory.

A. Albuquerque, M. Sousa

Centro de Medicina Laboratorial Dr Germano de SousaM (Portugal)

Background / Objectives

The spread of HIV epidemics globally has increasingly drawn attention to the interaction between HIV and the “classic” sexually transmitted infections (STIs). A consensus has grown that other STIs increase the spread of HIV, a hypothesis first suggested by Piot et al in 1984, following on from the early epidemiologic studies that explored the epidemiologic synergy between STIs and HIV.

However, the interaction of the many STIs with HIV is potentially complex, with the possibility of reciprocal influences on susceptibility, infectiousness, and the natural history of infections.

There is growing evidence of a significant burden of human papillomavirus (HPV) infection and associated disease in men.

HIV infection increases HPV prevalence, incidence and persistence and is strongly associated with the development of anogenital warts and anal, penile and head and neck cancers in men. Despite increasing access to antiretroviral therapy, there appears to be little benefit in preventing the development of these cancers in HIV-positive men, making prevention of infection a priority.

Methods

The authors present a 3 years revised casuistic as a reference laboratory center in sexually transmitted infection diseases diagnosis, focusing on HPV molecular methods such as Hybrid Capture 2 (hc2, Digene) , Cobas 4800 HPV test (16/18-Cobas, Roche), Clart human papillomavirus 2 (Genomica) and PapilloCheck used for HPV diagnosis in men as well as the diagnosis of “classic” sexually transmitted infections (STIs) as Herpes Simplex virus 1 and 2, Syphilis, Gonorrhea Chlamydia trachomatis, Ureaplasma and Mycoplasma infections.

Conclusion

The identified prevalence of anal HPV infection was high. Emerging patterns of HPV-related disease strengthen the call for universal vaccination of boys and girls with consideration of catch-up and targeted vaccination of high-risk groups such as MSM and those with HIV infection.

P12-02

Improving strategies and test algorithms for HPV diagnostics

F. Aliaga-Lozano¹, D. Jaén-Luchoro², M. Ruiz-Veramendi¹, A. Bennasar-Figueras²

¹Clínica Rotger (Spain), ²Universidad de las Islas Baleares (Spain)

Background / Objectives

Currently, international and national clinical guidelines recommend HPV testing on the screening of cervical cancer⁽¹⁾. This screening in Spain is advised for individuals between 21 and 65 years old⁽¹⁾. Such screenings are based on the triage of high-risk HPV and propose genotyping for HPV16 and HPV18, and cytology for women positive for any of the other 12 High-Risk (HR) genotypes⁽²⁾.

Clinical laboratories have a wide range of HPV based tests to decide the best option according to their necessities. The Xpert[®] HPV (GX-HPV) from Cepheid, and Anyplex[™] II HPV28 Detection (SG-HPV) from Seegene methods have proved excellent concordance (k 0.85, 95% CI 0.75 to 0.96) and a high agreement percentage; i.e., 92.93% (3).

The aim of this work is to present a testing algorithm, that is performed at the Clínica Rotger (CR) of Palma de Mallorca (Spain) as a routine protocol, for the diagnostic of HPV. The algorithm consists of a first-line-test with GX-HPV for information screening, and a second-line-test for genotyping with SG-HPV. Furthermore, they were compared for effectiveness and required time with a value stream mapping for the SG-HPV.

Methods

A total of 225 endocervical samples were collected and processed at the CR during 2015. The isolation and purification of nucleic acids for the SG-HPV method in DX Real-Time System (BioRad) was performed with the Virus minikit v2.0 (Qiagen). Moreover, the Cepheid based design does not require prior isolation and purification, since both steps are automatically included in the supplied GeneXpert cartridges system.

Results

The mean age of the patients included in the survey was 39.3 years old (95% CI 38.1 to 40.5); the 99.09% of the samples were included into the recommended age for the screening. The 61.36% of the results were negative (135/220); the samples positive for HPV16 reached 5.91% (13/220);

meaning while the 32.73% (72/220) were positive for at least one of other HR HPV, and consequently genotyped with the SG-HPV test; positive results for HPV18/45 were also tested with SG-HPV for individual typing. Remarkably, the first-line-test reduces a 57% hands-on time and it is 67% quicker than the second one.

Conclusion

The first-line-test (GX-HPV) provides results in less than 2 hours for “HPV16 positive” or “no detectable”. A second-line-test (SG-HPV) provides additional information about other HR and LR types.

The first-line test provides faster laboratory turnaround time, avoiding unnecessary genotyping of samples.

The second-line-test is basically useful for confirmation of doubtful results obtained along the first-line-test, gathering epidemiological information and for the analysis of samples where other HPV types have influence.

References

1. Aureli Torne Blade, Marta del Pino Saladrigues, Maite Cusido Gimferrer, Francesc Alameda Quitllet, Daniel Andia Ortiz, Xavier Castellsague Pique, Javier Cortes Bordoy, Rosario Granados Carreno, Rosa Maria Guarch Troyas, Belen Lloveras Rubio, Amina Lubrano Rosales, Juan Carlos Martinez-Escoriza, Jaume Ordi Maja, Luis M. Puig-Tintore, Mar Ramirez Mena, Silvia de Sanjose Llongueras, Rafael Torrejon Cardoso, Xavier Bosch Jose, Miguel Angel Piris Pinilla, Julio Rodriguez Costa, Rafael Comino Delgado, Josep M. Lailla Vicens y Jordi Ponce Sebastia, 2014. Guía de cribado del cáncer de cuello de útero en España, 2014. Rev Esp Patol. 2014;47(1):1-43. ISSN: 0031-3106.
2. Huh WK et al., 2015. Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance. Gynecol Oncol. Jan 6. pii: S0090-8258(14)01577-7. doi: 10.1016/j.ygyno.2014.12.022.
3. Aliaga-Lozano, F; Jaén-Luchoro, D; Bannasar-Figueras, A; Ruiz-Veramendi, M. Comparison of Xpert® HPV (Cepheid) with Anyplex™ II HPV28 Detection (Seegene) for the detection of High Risk Human Papillomavirus in clinical samples. Int J STD AIDS October 2015 26: 1-121, DOI: 10.1177/0956462415601768.

P12-03

XPert® HPV ASSAY EVALUATION FOR HPV TESTING ON FORMALIN FIXED PARAFFIN EMBEDDED OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS

F. Rollo¹, M.G. Donà², B. Pichi³, G. Spriano³, R. Covello¹, M. Benevolo¹

¹Regina Elena National Cancer Institute, Pathology Department (Italy), ²San Gallicano Dermatologic Institute, STI Unit (Italy), ³Regina Elena National Cancer Institute, Otolaryngology Head Neck Surgery Department (Italy)

Background / Objectives

Recent increase in incidence of HPV-related Oropharyngeal Squamous Cell Carcinoma (OPSCC) highlights the need for effective tools to evaluate cancer HPV status, also considering the improved survival and response to treatment for HPV-positive OPSCC. To this end, it is essential to have a simple and reliable method to detect high-risk (HR)-HPV types also in formalin-fixed paraffin-embedded (FFPE) tissues. Although there is no agreement regarding the most appropriate method for HPV testing on FFPE materials, the PCR-based INNO-LiPA genotyping assay is currently considered one of the most reliable assays due to the small length of the amplification fragment. The Xpert® HPV assay is a qualitative real-time PCR assay, validated to detect HR-HPV in cervical cytology, which also gives a partial genotyping result. This method is very fast and simple to perform, and could be also applicable in low resource settings. The aim of this study was to investigate the performance of the Xpert HPV assay on FFPE OPSCC samples, compared to INNO-LiPA HPV genotyping assay and p16ink4a immunostaining.

Methods

A series of 32 FFPE OPSCC samples, already analyzed by the INNO-LiPA HPV genotyping Extra assay (Fujirebio), was evaluated by Xpert HPV assay (Cepheid). Moreover, samples were immunostained for p16 (Roche Diagnostics), considered a biomarker of HPV transforming activity and, by many authors, a surrogate marker for HPV infection. Deparaffinization was carried out with xylene, followed by absolute ethanol washing, and tissue lysis was performed overnight in ATL buffer with Proteinase K (Qiagen). After 1 hour incubation at 90°C, half of the crude lysate was analyzed by the Xpert HPV assay.

Results

All the samples gave valid results with the Xpert, whereas two samples had been found invalid by the INNO-LiPA. These two samples were negative to both Xpert and p16 evaluations. Among the remaining 30 cases, 28 were concordant for HPV status, with a very high raw agreement and K value (93.3%; K=0.83). HPV genotyping results for the 21 cases positive by both methods, were also concordant. Regarding the two discordant cases, it is worth noting that one, Xpert positive/INNO-LiPA negative, was also p16 positive, whereas the other, Xpert negative/INNO-LiPA positive, was p16 negative. Comparing p16 and HPV results with the two methods, we found that the concordance rate with p16 staining was higher for Xpert than for INNO-LiPA (K= 0.92 vs K=0.73).

Conclusion

The Xpert HPV assay, a fast and easy method for HPV detection and partial genotyping, shows a very high concordance with INNO-LiPA genotyping assay and p16 findings, and appears to be reliable when used on FFPE OPSCC samples

P12-04

Audit of HPV subtypes in Northern Irish (NI) and Other Nationality (ON) patients with CIN111 biopsy

R. Clarke¹, S. Hegarty², K. Scott²

¹Craigavon Area Hospital, Lurgan Rd, Craigavon, BT65 8QQ, Northern Ireland (United kingdom), ²Institute of Pathology, Royal Group of Hospitals, Grosvenor Rd, Belfast BT12 9AB (United kingdom)

Background / Objectives

Between 2000 and 2010, an estimated 122,000 long-term, mainly Polish and Lithuanian immigrants arrived in Northern Ireland, 51% of whom are served by our cervical smear screening service. HPV triage for low-grade smears commenced in January 2013, results categorised as HPV16, HPV18 or "Other High Risk HPV subtype" (OHR HPV). A Pathology trainee had the impression that "other national" ON samples were more likely to be OHR HPV than HPV 16/18 positive, prompting this Audit.

Methods

Nationality was confirmed on a central screening database. We audited patients whose smear was low grade (LG), high risk HPV positive whose subsequent biopsy was CIN11 or more (116 indigenous NI and 13 ON). We also audited a smaller sample of smears reported as High Grade (HG) dyskaryosis (46 NI and 16 ON). The High Risk HPV Profile of each group was then determined.

Results

LG smear to CINIII biopsy: 43% of the NI group have OHR HPV detected , contrasting with 54% in the ON group. HG smear to CINIII biopsy: 29% of NI population have OHR HPV, contrasting with 39% in the ON group. Amalgamated results (HG + LG): 39% of NI group have OHR HPV, contrasting with 45% in the ON group. Interestingly the 2 cases of invasive carcinoma, both from NI patients in this audit, were OHR HPV detected. HPV results for biopsy proven CIN II – amalgamated (HG + LG) results: 57% of the NI population have OHR HPV, contrasting with 45% in the ON group.

Conclusion

The sample size in this audit is too small for rigorous statistical analysis. However they hint at an increase in prevalence of OHR HPV in the ON group, in which HPV 18 also appears lower. The proportion of OHR HPV, in both NI and ON groups in samples initially reported as High grade, CINIII biopsy +ve is much lower, raising a few interesting possibilities. Is disease associated with HPV 16/18 easier to recognise, or are there sampling issues in obtaining the smear? The audit highlights the degree of OHR HPV associated with precancer, CINIII. If confirmed, do these data have implications for the utility of HPV16/18 vaccination?

P12-05

EVALUATION OF THE MANAGEMENT OF HR-HPV+/PAPTEST-WOMEN: RESULTS AT 1-YEAR RECALL.

C. Chiappetta¹, C. Puggioni¹, E. Lendaro¹, J. Cacciotti¹, G. Migliore¹, P. Bellardini², V. Petrozza², C. Della Rocca², C. Di Cristofano²

¹UOC of Pathology, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome, Polo Pontino, I.C.O.T, Latina. (Italy), ²Screening Unit, Local Health Unit of Latina (Italy)

Background / Objectives

With cervical cancer screening the choice of 1-year as a period of follow-up in positive high-risk HPV women without cytological lesions is still under discussion. We evaluated the management of these women and the role of HPV genotyping test.

Methods

We did a cervical cancer screening study of women aged 35-64 with primary high-risk HPV test. Women positive for high-risk HPV with negative cytology were followed-up after 1 year. In this study we selected women with high-risk HPV+/PapTest- resulted high-risk HPV+ at recall and performed the PapTest and HPV genotyping test.

Results

The detection rate of squamous high grade (CIN2+) relative to the total screened cohort was 2.1‰, and it was 0.2‰ at the 1-year recall. The colposcopy performed in women referred at the 1-year recall accounted for 48.8% of the total (baseline + 1-year recall), and 84.3% of these women had no cytological lesions. The most frequent hr-HPV genotype detected was HPV16 and 66.7% of co-infections were due to HPV16 and HPV18. 54.5% of women presented a persistent infection at 1-year recall with the same HPV subtype, 50% of persistent infections was due to HPV16 and 16.7% of these were determined to be CIN2+ histological lesions.

Conclusion

Our data show that it may be useful to extend the period of follow-up for women hr-HPV+/PapTest- so as to reduce the number of unnecessary colposcopies due to the transitory infections and that the genotyping test could help to identify the persistent infections in which HPV16 is involved.

P12-06

IMPACT OF THE NEW MOLECULAR XPERT HPV ASSAY (CEPHEID) ON LABORATORY ORGANIZATION AND HPV MANAGEMENT

I. Thibaut, O. Guilhem

Pathology Center of Coteaux, Toulouse (France)

Background / Objectives

In the anatomy and cytopathology laboratory of Coteaux, we perform 40 000 Pap smears per year. We were using an in situ hybridization semi manual technique from Greiner to run HPV tests. This method was hogging two days of a full-time technician work. Technical difficulties to set up the assay were stress inducer for the team.

In 2014, Cepheid launched a new molecular assay (Xpert® HPV), which is a fully integrated assay (extraction, PCR and detection) with rapid and on-demand result. The issue with the first method was the necessary samples consolidation to run the batch, what was delaying responses compared with the Pap smear results, and prompted the change to the Xpert HVP assay.

Our purpose is to communicate about a two years' experience of Xpert HPV use.

Methods

The use of the Xpert HPV assay on the GeneXpert® system changed radically our routine habits and lab organization. The three PCR reactions are done inside the test cartridge; thereby the three rooms dedicated to PCR were not required anymore, simplifying the space management in the lab. By its easy-of-use, all technicians were trained and able to do the HPV test. The minimal hand of time (< 1 minute per sample) and the limited risk of errors and contamination reduced dramatically the technician stress. Xpert HPV is an on-demand test, with a time to result of 60 minutes, which allows us to provide the HPV testing result at the same time as Pap smear to gynecologists. This improved significantly the quality of our service for a better patient management and follow-up.

Xpert HPV report results are expressed as following: "Detected" or "Not detected" for the 14 HR HPVs. HPV types have been pooled in 5 channels, more one for the cellular control (HBMS gene).

P1: HPV 16

P2: HPV 18, 45

P3: HPV 31, 33, 35, 52, 58

P4: HPV 51, 59

P5: HPV 39, 56, 66, 68

SPC (HBMS).

Results

In 2015, we ran 1500 HPV tests (3.75 % of Pap smears), of which 1266 are performed after ASC-US (84.4%). Other tests are requested as part of post conisation surveillance and send-out demands from pathologists who are not able to do HPV testing in their lab.

Among the 1266 HPV tests, 412 were negatives (32.5%) and 854 were positives (67.5%).

The types distribution of the HPV positives was 29,9% (255), 14,4% (123), 41,5% (354), 26,7% (228) and 40,5% (346) for HPV 16, HPV 18/45, P3, P4 and P5 respectively.

Co-infections with 2 to 5 HPV types are frequent (additional data will be provide).

Conclusion

The new Xpert HPV assay has changed our HPV routine, with the benefits of simplicity, fast and on demand result, allowing us to develop our activity and get satisfaction from gynecologists, providing them a unique report for cytology and HPV testing.

P12-07

Population-based HPV Testing Performance: Potential False-negative HPV Results in a Cervical Cancer Surveillance Population

H. Zhou, R. Bassett, A. Khanna, M. Dawlett, N. Sneige, K. Schmeler, A. Milbourne, T. Bevers, Y. Gong, G. Staerke, M. Guo

MD Anderson Cancer Center (United States of America)

Background / Objectives

Human papillomavirus (HPV) testing has been recommended for primary cervical cancer screening in the United States. HPV testing performance in different patient populations has not been systemically evaluated.

Methods

We retrospectively searched our institution's database for women aged 30 years and older who underwent HPV/Pap cytology co-testing in our Cancer Prevention Center (CPC, for general screening population) or Gynecology Clinics (GYN, for cancer surveillance) (2007-2014). SurePath Pap tests and Hybrid Capture 2 or Cervista HPV assays were used in both centers. A total of 22,005 cases were analyzed, including 13,951 from CPC (mean age, 54.9 years; 30-91 years) and 8,054 from GYN clinics (mean age, 50.9 years; 30-96 years). HPV testing results were compared between the two populations. The differences were analyzed by the Fisher exact test.

Results

Women with abnormal Pap test results (\geq ASC-US) accounted for 6.5% in the CPC and 27.2% in the GYN clinics (Table 1). The distribution of HPV positivity is illustrated in Table 2. The overall HPV-positive rates were significantly different in women tested at the CPC (5.7%) vs. the GYN clinics (14.8%) ($P < 0.0001$). Pap tests with a high-grade squamous intraepithelial lesion (HSIL) or squamous carcinoma result were positive for HPV in 100% (21/21) of cases from CPC and 82.8% (130/157) of cases from GYN clinics ($P = 0.05$). In 25 women from the GYN clinics with HSIL/squamous carcinoma Pap cytology and a negative HPV result, the follow-up biopsy showed dysplasia in 20 cases, including high-grade dysplasia or squamous carcinoma in 14.

Table 1. Pap Cytologic Diagnosis in Women Screened at the Cancer Prevention Center (CPC) or Gynecology Clinics (GYN)

	NILM (%)	AGC (%)	ASC-US (%)	LSIL (%)	ASC-H (%)	HSIL/SqCa (%)	Total (%)
CPC	13044 (93.5)	55 (0.4)	589 (4.2)	199 (1.4)	43 (0.3)	21 (0.2)	13951 (100)
GYN	5866 (72.8)	150 (1.9)	1311 (16.3)	463 (5.7)	107 (1.3)	157 (1.9)	8054 (100)

Table 2. Positive HPV Test Rates in Populations at the Cancer Prevention Center (CPC) or Gynecology Clinics (GYN)

	NILM	AGC	ASC-US	LSIL	ASC-H	HSIL/SqCa
CPC HPV + (%)	3.8*	3.6	20.2	67.3	37.2	100**
GYN HPV + (%)	6.8*	9.3	21.9	68.5	39.3	82.8**

* P < 0/0001; ** P = 0.05

Conclusion

HPV testing performance for cancer screening is population dependent. Potentially higher false-negative HPV test results can occur in a cancer surveillance population compared to a cancer screening population. Further study is required to determine the cause of false negative HPV test results in cancer surveillance population.

P12-08

Distribution of high risk HPV genotypes in patients referred from medical offices in the Rhône-Alpes area (France)

M. Labadie, J. Depardon, C. Donne, J.F. Denier, B. Descombes, C. Lauro-Colleaux, D. Maisonneuve, H.P. Morel

Technipath (France)

Background / Objectives

In France, cervical cytology is used as the principal screening test to detect cervical cancer in asymptomatic women. The French Health Authority (HAS) recommends cervical cytology screening every three years (between 25 and 65 years of age) after 2 annual smears have been normal.

In 2015, we have adopted a new technology for HPV testing the Xpert® HPV assay used on the GeneXpert® system (Cepheid, Sunnyvale, USA).

We are now performing routine HPV testing for women ages 17 to 85 years who present abnormal cervical cytology: ASCUS and LSIL.

Methods

This study was conducted between January 1st 2015 and December 31st 2015 in the anatomico-cytology laboratory Technipath (Limonest, France). HPV genotyping was performed on 4980 specimens collected into Thinprep (Hologic) collection vials, using real-time PCR with Xpert HPV which allows the detection in one hour of the 14 high-risk HPV types, using 5 fluorescent channels either separated or combined: HPV 16; HPV18 and 45; HPV 31-33-35-52-58; HPV 51, 59; HPV 39-56-66-68. The 335 health care practitioners who prescribed the HPV test were physicians, gynecologists, obstetricians or mid-wives located in the Rhône-Alpes area in France.

Results

Among the 4980 samples tested, 2876 samples were negative for high risk HPV types and 2098 samples presented at least one oncogenic high-risk HPV type :

HPV 16: 527 (25.12 %)

HPV 18, 45: 207 (9.7 %)

HPV 31-33-35-52-58: 750 (35.75 %)

HPV 51-59: 275 (13.11 %)

HPV 39-56-66-68: 339 (16.16 %)

6 samples were excluded due to insufficient cellularity. Indeed, Xpert HPV has a sample adequacy control (SAC) that monitors whether the sample contains human DNA and eliminates the risk of false negative results.

Among the infected patients in 2015, 32.6 % were younger than 30 and 67.4 % were over 30. The mean age was 37 years. For the group under 30, the mean age was 25 years and for the group over 30 it was 42 years.

Conclusion

In our patient population, the infections caused by HPV other than 16 and 18 are the most frequent ones (65%). The positivity rate (i.e. cases with at least one high risk HPV type detected) was 58.96 % (1160 cases) before 30 years of age and 37.07 % (3814 cases) after 30 years of age. There is no significant difference in the mean age according to the HPV genotype. Likewise we did not observe any significant difference in the mean age when a single channel is positive (1504 cases) or when multiple channels are positive (1191 cases).

With Xpert HPV routinely used in our laboratory, we are now able to provide rapid comprehensive HPV results to effectively risk stratify patients based on cytology and high risk HPV.

P12-09

PERFORMANCE OF COBAS 4800 TEST TO DETECT HRHPV IN HSIL+ LESIONS

M.L. Mateos ¹, A. Suarez ¹, M.P. Dominguez ², A. Benito ², B. Perez-Mies ²

¹Microbiology Department. H Ramón y Cajal. Madrid (Spain), ²Pathology Department.H. Ramón y Cajal. Madrid (Spain)

Background / Objectives

The cobas[®] 4800 HPV Test (Roche Molecular Systems, Inc., Branchburg, NJ, USA) is an automated qualitative in vitro test for the detection of 14 high risk HPV genotypes. This system identifies HPV16 and HPV18 genotypes separately, while simultaneously detecting 12 other HRHPV (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) at clinically relevant infection levels in cervical samples. In the present study we aimed to correlate the detection of HRHPV by cobas[®] 4800 with pre malignant cervical lesions.

Methods

From January to December 2014, 145 cervical samples from 145 women attending the Gynaecology Unit with a result of HSIL lesions in cytology were included in this study. Biopsy data were also analyzed. The presence of HR HPV were investigated by cobas[®] 4800. The same vial (Thin Prep) was used for cytology and HPV detection

Results

BIOPSIA	16	18	HRHPV	16+HRHPV	18+HRHPV	16+18	16+18+HRHPV	NEGATIVE	TOTAL
NILM	0	0	2	0	0	0	0	2	4
CIN1	0	0	4	2	0	0	0	0	6
CIN2	3	1	15	8	2	0	0	1	30
CIN3	3	3	26	17	2	1	1	2	82

	0								
CANCER	0	0	1	1	0	0	0	0	2
METAPLASIA A	1	0	4	4	0	0	0	1	10
NO BIOPSY	3	0	6	2	0	0	0	0	11
TOTAL	3 7	4	58	34	4	1	1	6	145

Conclusion

Biopsy data were not available for 11 out of the 145 patients. 10 cervical samples were informed as squamous metaplasia. 4, 6, 30 and 82 cervical samples were reported as NILM, CIN1, CIN2 and CIN3 respectively and 2 invasive cancers were found.

HPV16 and 18 as a unique genotype was present in 37 and 4 samples, respectively. HPV16 and HPV18 together were detected in 1 sample. Mixed infection including HPV16 plus HRHPVnon16,18 and HPV18 plus HRHPVnon16,18 were present in 34 and 4 samples, respectively. HPV16 plus HPV18 plus HRHPV non16,18 in only 1 sample. HRHPV non16,18 were found in 58 samples. 6 samples had negative results for any HRHPV. The histology results of these 6 negative samples were 2 NILM, 1 squamous metaplasia, 1 CIN2 and 2 CIN3.

Cobas 4800 detected the presence of HRHPV in 139 of 145 histologically confirmed HSIL+ samples. HPV16 and/or HPV18 were present and differentiated separately in 81 (58.2%) samples, in 41/81 (50.6%) as a unique genotype. To identify separately HPV16 and HPV18 is important since these women even with normal cytology should be referred for colposcopic examination

P12-10

BARRIERS TO AND FACILITATORS OF HPV SELF-TESTING AMONG WOMEN POST TREATMENT OF HIGH GRADE CIN

E. Östensson¹, M. Minst², S. Andersson²

¹Department of of Women's and Children's Health, Karolinska Institutet, and Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (Sweden), ²Department of of Women's and Children's Health, Karolinska Institutet, Stockholm (Sweden)

Background / Objectives

Women treated for high grade cervical intraepithelial neoplasia (CIN) have an increased risk of recurrence of disease and cervical cancer (1, 2). Swedish cervical cancer prevention program recommend that women treated for high grade CIN should have colposcopic examination and cytology test every 2d year for 25 years. Human papillomavirus (HPV) testing with self-collected vaginal sampling (“HPV self-testing”) is one suggested strategy as test-of-cure (ToC). HPV self-testing is performed outside the healthcare provider’s office and may therefore increase accessibility to follow-up by reducing barriers such as privacy, patient costs or provider’s availability. To succeed, programs for HPV self-testing need to overcome disparities in knowledge and perceptions related to HPV, self-testing and cervical cancer prevention. In Sweden, HPV self-testing will not replace colposcopic examination, but rather may initiate the follow-up process and facilitate the early detection of recurrence of high grade CIN. **Objectives:**To identify possible barriers to and facilitators of HPV self-testing by (a) assessing women’s perceptions of self-testing, knowledge of HPV and perceived risk of cervical cancer (b) estimating costs incurred by women attending the clinic-based follow-up procedure (c) investigating their vaccination status and its impact on residual/recurrent HPV infection and (d) examining correlates of HPV knowledge and perceptions of self-testing.

Methods

Data on sociodemographic characteristics, cost for time and travel and other direct non-medical costs incurred in attending gynecology follow-up (e.g., indirect cost of time needed for the visit, transportation costs, child care costs, etc.), mode(s) of travel, distance, companion's attendance, knowledge of HPV and related diseases, perceptions of HPV self-test (self-collection of vaginal fluid), perceived risk of cervical cancer and vaccination status are obtained via self-administered questionnaires. For data analysis, we will use logistic and linear regression to assess bivariate associations between sociodemographic characteristics and measures related to self-testing and knowledge of HPV and cervical cancer prevention.

Results

Data has hereby been collected from half of the 500 women included in the study population attending gynecological follow-up 6 months post first life-time treatment of high grade CIN. Results from the data analysis will be presented at the conference.

Conclusion

To fully grasp the potential of HPV self-testing as ToC, healthcare providers and decision makers for the cervical cancer prevention program should be aware of the levels of HPV knowledge and perceived risk related to cervical cancer.

References

1. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine*. 2006;24 Suppl 3:S3/78-89
2. Strander B, Andersson-Ellstrom A, Milsom I, Sparen P. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. *BMJ*. 2007;335(7629):1077.

P12-11

Comparison of the HPV DNA chip test, the real time PCR HPV test and the Hybrid capture II test in Atypical Squamous Cells of Undetermined Significance population

Y.K. Lee, J.S. Park

Department of Obstetrics and Gynecology, Seoul St. Mary's Hospital, The Catholic University of Korea (Korea, republic of)

Background / Objectives

The sensitivity of the HPV test for detecting cervical cancer and its precursor lesions has been reported to be far higher than Pap smear test, and it is now recommended for use in “cotesting” with Pap smear test in some parts. Accordingly, the accuracy analysis followed by the quality control of each HPV test are demanded for the appropriate diagnosis and treatment. This study was carried out to compare the performance of HPV DNA chip test, real time PCR HPV test and Hybrid capture II test in ASCUS population.

Methods

We performed HPV DNA chip test, real time PCR HPV test and Hybrid capture II test in 504 cervical swab samples of ASCUS patients diagnosed in 5 hospitals, from February 2012 to August 2014. The concordance rates between the three tests, including those detecting HPV 16 or 18 in case of HPV DNA chip test and real time PCR HPV test, finally the result of sole discrepancy of each test were analyzed.

Results

The concordance rate between real time PCR HPV test (PCR) and Hybrid capture II test (HCII) was 86.3%, HPV DNA chip test (Chip) and HCII was 84.9%. In terms of detecting HPV 16 or 18, the concordance rate between PCR and Chip was 97.2%, however, overall concordance rate of identifying the existence of HPV between above two tests was 79.2%, and slightly higher to 80.8% when only high risk HPV types were categorized as positive result in Chip. On analysis of only Chip negative results (n=62), when negative was defined as the absence of high risk types, 67.7% (42/62) of the cases were “other” types on Chip. On the other hand, 50% (10/20) of the cases where PCR showed sole negative result (n=20), Chip-detected HPV types were either HPV 68 (n=8) or HPV 58 (n=2). The other half of PCR-undetected HPV types were those not included in the test system. There

was only one case where HCII showed sole negative result, and among 35 cases of HCII sole positive result, 65.7% (23/35) were in HCII RLU/CO level gray zone (1~10).

Conclusion

HPV DNA chip test may be as accurate as real time PCR HPV test when detecting HPV 16 or 18, which accounts for approximately 70% of all invasive cervical cancer. However, when the result of HPV DNA chip test shows HPV other types, subsequent meticulous observation or trial of different HPV test are of concern, as the sole discrepancy may be numerous. Additionally, it would worth to keep in mind that real time PCR test is vulnerable in detecting HPV 68 or 58. Finally, careful interpretation would be required for those of the results in RLU/CO level gray zone in Hybrid capture II test.

P12-12

EVALUATION OF ONCOGENIC HUMAN PAPILLOMAVIRUS RNA AND DNA TEST WITH LIQUID-BASED CYTOLOGY IN PRIMARY CERVICAL CANCER SCREENING POPULATION.

A. Sáez¹, C. Garcia-Andrade², I. Gallego², I. Romero³, P. Cano³

¹. Servicio Laboratorio Central. Microbiología. Hospital Universitario Santa Cristina. Madrid. Spain. (Spain), ². . Servicio Anatomía Patológica. Hospital Universitario Santa Cristina. Madrid. Spain. (Spain), ³.. Servicio Ginecología y Obstetricia. Hospital Universitario Santa Cristina. Madrid. Spain (Spain)

Background / Objectives

Background / Objectives *

The aim of this study is to evaluate the performance of the Cobas 4800 HPV test and Aptima HPV Assay by comparing their results to those obtained in the Hybrid Capture 2.

Methods

We have studied 736 cervical samples, which were obtained from women attending gynecology practitioners, in the cervical pathology unit from our hospital, in a routine cervical cancer screening program.

All specimens were collected with PreservCyt transport medium.

Each sample was analyzed with Cobas 4800 HPV (Roche Molecular System, Inc), E6/E7 mRNA-based Aptima® HPV(AHPV; (Hologic, Inc) and Hybrid Capture 2 HPV DNA test (HC2; Qiagen).

In each patient we made Pap smears, and byopsie an p16 when the patient required it.

Statistics analyses were done with SPSS 18 for windows.

Results

The average age was 38.02 (19-90).

The prevalence of HPV in each test is shown in Table 1

HPV	HC2	DNA Cobas 4800	mRNA AHPV
Positive	44,4% (327)	50,1% (369) p=0.032	41,0% (302)
Negative	51,9% (382)	49,9% (367)	59,0% (434)

Kappa value DNA Cobas 4800 = 0.834

Kappa value mRNA AHPV =0.805

Our Pap smear distribution, and the frequency of HPV in each category is shown in Table 2

Cytology	% HC2 Positive	%Cobas 4800 Positive	% APTIMA Positive
Negative (n=450)	26,8	28,4	20,4
ASCUS (n=56)	66,0	75,0	62,5
LSIL (n=108)	81,4	85,1	74,1
HSIL (n=112)	88,3	91,9	84,0

203 patients had a CIN2+ biopsy result and for HPV, the results obtained were: HC2 (90,3%), Cobas 4800 (93,8%) and AHPV 88,5%. There were no statistically significant differences in frequency of HPV between techniques.

p16 made in 483 biopsies and the results were associated more frequently with the samples mRNA positives: 78,01% versus 75,1% for both DNA techniques.

We calculated the sensitivity and specificity for both techniques:

mRNA E6/E7 AHPV sensitivity = 0.83 [95% CI: 0.79-0.87] specificity = **0.99** [95% CI: 0.94-0.99]

Cobas 4800 DNA sensitivity = 0.94 [95% CI: 0.91-0.96] specificity = 0.90 [0.95% CI: 86-0.92]

Conclusion

Hybrid Capture 2, Cobas 4800 HPV and Aptima HPV Assay showed a high degree of agreement in the results obtained.

In our data, similar sensitivities of Aptima HPV Assay and Hybrid Capture 2 were observed and in Cobas 4800 HPV the sensitivity was higher (p=0.032).

The better specificity of Aptima HPV, support the use of Aptima mRNA as a safe and effective adjunctive cervical cancer screening method.

References

- [1. Basu P, Banerjee D, Mittal S, Dutta S, Ghosh I, Chowdhury N, Abraham P, Chandna P, Ratnam S. Sensitivity of APTIMA HPV E6/E7 mRNA test in comparison with hybrid capture 2 HPV DNA test for detection of high risk oncogenic human papillomavirus in 396 biopsy confirmed cervical cancers. *J Med Virol.* 2015 Dec 22.doi:10.1002/jmv.24453]
- [2. Haedicke J(1), Iftner T(2). A review of the clinical performance of the Aptima HPV assay. *J Clin Virol.* 2015 Nov 6. pii: S1386-6532(15)00736-2. doi:10.1016/j.jcv.2015.10.027]
- [3. Nakayama Y, Yamada M, Kurillomavirus mRNA test for the detection of cervical lesions in Japan. *Eur J Gynaecol* ata A, Kiseki H, Isaka K, Kuroda M. Evaluation of the human papOncol. 2015;36(2):192-6]

P12-13

THE IEO LAB TRANSITION FROM HC2 TO COBAS IN HPV DETECTION: SENSITIVITY AND SPECIFICITY FOR CIN2+ IN 10213 CONSECUTIVE SAMPLES.

F. Bottari¹, S. Boveri², S. Igidbashian², F. Landoni², M.T. Sandri²

¹Division of Laboratory Medicine, European Institute of Oncology, Milan, Italy (Italy), ²Preventive Gynecology Unit, European Institute of Oncology, Milan, Italy (Italy)

Background / Objectives

High Risk (HR) Human Papilloma Virus (HPV) Tests for HPV detection differ in sensitivity and specificity. In this study we evaluated the sensitivity and specificity of Qiagen HC2 HR HPV Test® (Qiagen) and Roche Cobas 4800 HPV Test® (Roche Diagnostics) in consecutive cervical samples collected from a referral population with a high prevalence of disease, using CIN2+ histology as clinical outcome. High Risk (HR) Human Papilloma Virus (HPV) Tests for HPV detection differ in sensitivity and specificity. In this study we evaluated the sensitivity and specificity of Qiagen HC2 HR HPV Test® (Qiagen) and Roche Cobas 4800 HPV Test® (Roche Diagnostics) in consecutive cervical samples collected from a referral population with a high prevalence of disease, using CIN2+ histology as clinical outcome.

Methods

10213 consecutive cervical samples were assayed for HR-HPV for any reason in Laboratory Medicine Division of IEO: 5140 from January 2012 to June 2013 with HC2 and 5073 from July 2013 to December 2014 with Cobas HPV Test. These two assays differ in terms of target genes and testing methods: HC2 is a signal amplification detection system based on chemiluminescence that detects 13 HR-HPV, while Cobas HPV test is a fully automated real-time PCR assay that detects the same 13 HR genotypes of HC2 plus HPV 66, and allows, in case of positive results, to differentiate HPV 16 and 18 from the other HR HPV genotypes.

Results

Overall in the three years under consideration we found 223 CIN2+: in the first period we diagnosed 144 CIN2+, of which 24 were HC2 negative (16.4%); in the second period we diagnosed 79 CIN2+ of which 13 were Cobas HPV negative (16.5%). In our population no statistically significant difference in terms of sensitivity and specificity of HC2 and Cobas was found.

Conclusion

The analysis of the performance of HC2 and Cobas HPV testing in the detection of CIN2+ lesions in a referral population showed comparable performance of the two test. The difference in sensitivity and specificity of a test should be considered when choosing the HPV test to use in routine: in our experience we did not find significant changes in sensitivity and specificity between HC2 and Cobas 4800 in CIN2+ detection.

P12-14A

COMPARISON BETWEEN LIQUID BASED CYTOLOGY AND HUMAN PAPILLOMAVIRUS DETECTION AS TESTS FOR CERVICAL CANCER SCREENING.

M.E. Mustafa¹, A. Trauner¹, D. Rieder¹, M. Weik¹, C. Obermoser¹, A. Kirnstedter¹, A. Graf², G. Mustafa¹, A.H. Graf³

¹MEDILAB, Salzburg (Austria), ²Ordination Graf Angelika Dr. Med. Univ., Salzburg (Austria), ³Cytological Diagnostic Laboratory Graf Anton-H. Univ.-Doz. Dr.med.univ., Salzburg (Austria)

Background / Objectives

Between 2013 and 2015 a total of 1186 liquid based samples were collected from 750 women with conspicuous clinical data with or without primary abnormal PAP test. The aim of this study was to compare the PAP test and the HPV detection test for timely discovery of cervical cancer and its precursors.

Methods

Samples for the PAP test and for the detection of high-risk HPV were taken from the same liquid based material. The PAP test was based on liquid based cytology (LBC). The LBC samples were prepared according to the BD working protocol (Becton, Dickinson and Company). The molecular biological detection of high-risk HPV was determined by PCR (cobas® HPV Test; F.Hoffmann-La Roche AG), that provides specific genotyping information for HPV Types 16 and 18. At the same time 12 other high-risk HPV types were detected in a pooled result. For this study we called the negative result for intraepithelial lesions or malignancy (PAPI and PAPII) a negative PAP test. The results PAPIII; PAPIIIG; PAPIIID and higher: positive PAP test. The results were evaluated as statistically significant at $p < 0,05$ (NCSS Statistical Software).

Results

The average age of the 750 tested women was 44,8 (between 15 and 89). There was a high statistically significant difference ($p=0,00000$ chi-square test) between positive/negative results (65/685) of the PAP test and positive/negative results (137/613) of high-risk HPV detection. 102 of 750 tested women (13,6%) had a positive result concerning high-risk HPV detection but a negative PAP test. The women with negative cytology results were positive for HPV 16 in 2,0% of all cases (15/750) and for at least one of the 12 other high-risk HPV subtypes in 9,1% of all cases (68/750).

1,9% of the women with a negative PAP test had a mix of different subtypes of the tested high-risk HPV (14/750). All women (n=5) infected with only HPV 18 had a negative PAP test result (0,7%).

Conclusion

The gynecological examination represents an important contribution to women's health and allows diagnostics, therapy but also counselling and prevention. Nowadays the HPV test is conducted to clarify the primary abnormal PAP test in routine check-ups. Our study shows that it would be better to establish the molecular biological test as primer test, because the test can better provide information about the patient's risk for developing cervical cancer in the future. Cytology should be used to diagnose cervical cancer and its precursors.

P12-14B

THE CORRELATION BETWEEN THE MOLECULAR BIOLOGICAL DETECTION OF HUMAN PAPILLOMA VIRUS AND THE GRADE OF CERVICAL NEOPLASIA.

M.E. Mustafa, A. Trauner, D. Rieder, S. Legner, C. Schausberger, G. Mustafa

MEDILAB, Salzburg, Austria (Austria)

Background / Objectives

1055 reports of cone biopsy (between 2013 and 2015) had been screened for previous results of HPV detection. The aim of this study was to observe and evaluate the correlation between the reports of high-grade human papilloma virus and the reports of cone biopsy in follow-up.

Methods

Each histology report was compared with outcomes of high-risk HPV: HPV 16 and HPV 18 specific genotyping and the pooled result of HPV Types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (cobas® HPV Test; F.Hoffmann-La Roche AG). The reports of cone-biopsy were evaluated in four groups: negative for squamous intraepithelial lesions or malignancy (negative), low grade squamous intraepithelial lesions (LGSIL), high grade squamous intraepithelial lesions (HGSIL) and invasive carcinoma. The results of the comparison were evaluated as statistically significant at $p < 0,05$ (NCSS Statistical Software).

Results

342 women (average age 36,1 years) had been tested for high-risk HPV before. The average period between the last HPV typing and the conisation was 4,1 months. The report of cone biopsy was: 5,3% negative cases (18/342), 19,0% LGSIL cases (65/342), 74,3% HGSIL cases (254/342) and 1,5% cases of invasive carcinoma (5/342). 302 out of the 342 women with cone biopsy (88,3%) were positive according to the high-risk HPV test. There was a high statistically significant difference ($p=0,00000$; Chi -square test) between negative/positive HPV results in relation to the grade of cervical intraepithelial lesions in follow up. The distribution negative/positive HPV results in women with low grade squamous intraepithelial lesions was 19/46 compared to 14/240 negative/positive cases in women with high grade squamous intraepithelial lesions.

Conclusion

The different frequency of high risk HPV positivity in women with a cone biopsy report clearly reflect the correlation between the type of detected HPV and the grade of lesion in follow up. Women with low grade intraepithelial lesion were high-risk HPV positive in 70,8% cases (46/65). The percentage increases to 94,5% in women with high grade intraepithelial lesion (240/254). As expected all women with invasive carcinoma were high-risk HPV positive. Molecular biological testing for high-risk human papillomavirus provides information about the patient's risk for developing cervical lesions in follow up.

P12-15B

AN ANALYSIS OF RISK ASSESSMENT CONCERNING THE GENESIS OF CERVIX CARCINOMA.

A. Trauner¹, D. Rieder¹, C. Obermoser¹, A. Kirnstedter¹, C. Schausberger¹, A.H. Graf², G. Mustafa¹, M.E. Mustafa¹

¹MEDILAB, Salzburg (Austria), ²Cytological Diagnostic Laboratory Graf Anton-H. Univ.-Doz. Dr.med.univ.,Salzburg (Austria)

Background / Objectives

Nowadays the HPV test is conducted to clarify conspicuous clinical data or primary abnormal PAP-test. For research of the timely risk assessment at the genesis of cervix carcinoma we compared retrospective the conventional cytology and the HPV detection in the routinely screening.

Methods

Between June and December 2012 a total of 32261 women had a cytological result by conventional cytology from our laboratory. For this study, we investigated the cytological result, which was supplemented with high-risk HPV typing result (cobas[®] HPV Test; F.Hoffmann-La Roche AG) from the same laboratory within one year. The results of conventional cytology were grouped in four categories: negative for intraepithelial lesions or malignancy (NILM), low grade intraepithelial lesions (LSIL), high grade intraepithelial lesions (HSIL) and atypical squamous cells of undetermined significance (ASC-US). The results of the comparison were evaluated as statistically significant at $p < 0,05$ (NCSS Statistical Software).

Results

1,2% of the women (n=376) had a cytology report accomplished with high-risk HPV result within one year. The average period between cytology results and HPV typing was 2,1 months. The mean age of the 376 women was 39,6 years (between 16 and 84 years). 72,3% of the women had a result negative for intraepithelial lesions or malignancy (272/376). 65,7% of the women were high risk HPV negative (247/376). There was a statistically significant difference ($p=0,04867$; Chi-Square test) between the positive/negative results of conventional cytology and the positive/negative results of high-risk HPV detection (104/272 to 129/247). More than half of the women were negative for both tests (n=211; 56,1%). 68 women were positive for both tests (18,1%). 36 women had a positive cytological report (5 ASC-US cases and 31 LSIL cases) and were high-risk HPV negative (9,6%). 16,2%

of the women (n=61) were high-risk HPV positive but there were no intraepithelial lesions registered

Conclusion

As expected all women with high grade intraepithelial lesions were high-risk HPV positive. The high-risk HPV negative women with reports of low grade intraepithelial lesions or atypical squamous cells of undetermined significance were probably infected with other HPV types (low-risk HPV is most to be expected). The high incidence of high-risk HPV positivity in women with negative cytology report clearly shows the utility of HPV typing for the objective risk assessment concerning the genesis of the cervix carcinoma.

P12-16

PILOT FEASIBILITY STUDY ON USE OF THE XPERT HPV ASSAY WITH COLLI-PEE COLLECTED, UCM PRESERVED URINE.

A. Vorsters¹, **V. Vankerckhoven**², **S. Van Keer**¹, **S. Biesmans**¹, **K. Beyers**², **M. De Koeijer**², **H. Revets**¹, **P. Van Damme**¹

¹University of Antwerp, VAXINFECTIO (Belgium), ²Novosanis (Belgium)

Background / Objectives

HPV testing in urine has been proposed for monitoring impact of vaccination, follow-up of treatment and/or reaching women not participating in a cervical cancer screening programme. The use of Colli-Pee™ (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine. The Xpert® HPV assay (Cepheid, Sunnyvale) has been validated to detect HPV DNA in cervical samples, but no data are currently available regarding HPV DNA detection in urine.

The aim of this study was to determine if the Xpert HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.

Methods

Fifteen Colli-Pee™ collected, UCM preserved urine samples were analysed. These samples originated from a cohort of women participating in a therapeutic HPV vaccination trial and were characterised by an in-house HPV type specific (TS) qPCR method (UAntwerp, Belgium). The samples were collected by the participants at home and were send uncooled by mail to the University of Antwerp.

Results

This pilot study demonstrates that the GeneXpert® platform (Cepheid, Sunnyvale) performs well with the Colli-Pee™ collected, UCM preserved urine samples. HPV DNA was detected by the Xpert HPV assay in most of the HPV positive samples.

A correlation between the Ct (cycle threshold) values obtained with Xpert HPV and the in-house TS qPCR is observed for human DNA and HPV DNA. This further confirms the compatibility of the Cepheid platform and Colli-Pee™ collected, UCM preserved urine. We did find some indications of a lower analytical sensitivity for HPV DNA, but for screening purposes this may even be an advantage

to obtain the required clinical performance. All samples with a Ct value below 35 in our in-house TS qPCR for HPV 16 and/or 18 were also positive with the Xpert HPV assay.

Conclusion

These results are very encouraging to further investigate the performance of first void collected, UCM preserved urine in combination with Xpert[®] HPV. Especially, as both the collection and the detection system can function outside the cold chain, this may lead to innovative HPV testing opportunities in low-resource and point-of-care settings.

P12-17

EVALUATION OF THE ROCHE COBAS®HPV ASSAY WITH COLLI-PEE™ COLLECTED, UCM PRESERVED URINE.

A. Vorsters¹, S. Van Keer¹, S. Biesmans¹, V. Vankerckhoven², M. De Koeijer², K. Beyers², L. Ceelen³, A. Van Roost⁴, P. Van Damme¹

¹University of Antwerp, VAXINFECTIO (Belgium), ²Novosanis (Belgium), ³Anacura (Belgium), ⁴Roche (Belgium)

Background / Objectives

HPV testing in urine has been proposed for monitoring impact of vaccination, follow-up of treatment and/or reaching women not participating in a cervical cancer screening programme. The use of Colli-Pee™ (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine. The Roche Cobas®HPV assay has been validated for clinical sensitive during the ATHENA trial enrolling > 47000 women on cervical material.

The aim of this study was to determine if the Roche Cobas® HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.

Methods

Forty-four Colli-Pee™ collected, UCM preserved urine samples were analysed. Thirty-two of these samples originated from a cohort of women participating in a therapeutic HPV vaccination trial. These samples were collected by the participants at home and were send uncooled by mail to the University of Antwerp. All samples were characterised by an in-house HPV type specific (TS) qPCR method (UAntwerp, Belgium) and or by the Optiplex HPV genotyping kit (Diamex).

Results

All samples were positive for the Roche beta-globin internal control.

If we look for HPV16 or HPV18 positivity, 20 samples were concordant negative, 14 concordant positive, and 10 were Diamex positive and Cobas®HPV negative. However, the discrepant results were obtained in samples with low MFI values, and low HPV copy numbers. In addition, the Diamex assay and the in-house PCR are performed on DNA extracts obtained after ultra-filtration of the urine sample, therefore these assay are run with DNA enriched samples compared to the Cobas®HPV.

A correlation between the Ct (cycle threshold) values obtained with Cobas® HPV and the in-house TS qPCR is observed for human DNA and HPV DNA. This further confirms the compatibility of the Roche assay and the Colli-Pee™ collected, UCM preserved urine.

Conclusion

This pilot study demonstrates that the Roche Cobas® HPV assay is compatible with the Colli-Pee™ collected, UCM preserved urine samples. These results are very encouraging to further investigate possible applications of first void urine in combination with Cobas® HPV assay.

P12-18

HPV-RELATED LESIONS IN ANAL AND CERVIX PAP-SMEARS IN HIV+-PATIENTS UNDER ANTI-RETROVIRAL TREATMENT. INTEREST OF CYTOLOGY COUPLED TO RAPID HPV PCR.

H. Nassereddine ¹, L. Bienvenu ¹, L. Deschamps ¹, C. Davitian ², L. Abramowitz ³, D. Benabderrahmane ³, A. Couvelard ¹, C. Charpentier ⁴, T. Lehy ¹, F. Walker ¹

¹(1) Pathology Department Hospital Bichat Claude Bernard, 46 rue Henri Huchard, 75877, Paris, France (France), ²(2) Gynecology Department, SMIT Hospital Bichat Claude Bernard, 46 rue Henri Huchard, 75877, Paris (France), ³(3) Proctology Department, Hospital Bichat Claude Bernard, 46 rue Henri Huchard, 75877, Paris (France), ⁴(4) Virology Department, Hospital Bichat Claude Bernard, 46 rue Henri Huchard, 75877, Paris (France)

Background / Objectives

Prevalence of low (LSIL) and high (HSIL) grade squamous intraepithelial lesions with oncogenic-HPVs (Human Papilloma Virus) was studied in successive HIV (Human Immunodeficiency Virus) + patients under anti-retroviral treatment. This was done in anal and/or cervical Pap smears with rapid PCR technique detection of oncogenic-HPVs types.

Methods

Anal and/or cervical cytologies (Ilsa technique) were obtained under colposcopy and/or anoscopy in 238 subjects recruited during 6 months. Bethesda classification was associated with Rapid PCR (Cepheid) to detect HPV16, both HPV18/45, P3 (HPV31,35,33,52,58), P4 (HPV51,59), P5 (HPV39,68,56,66).

Results

59 anal smears were obtained in 48 patients: 42 men (88%) and 6 women (12%) (10 patients had several anal smears or both margin and canal samples). Median age was 42 years (26-72). Oncogenic-HPVs prevalence was 73% (N=35 patients). P3 group slightly predominated in patients (N=22 patients; 22 smears) while HPV16 was present in 23 anal smears (N=17 patients). In fact, mutiviral lesions existed in 21 smears (N=19 patients) exhibiting frequently HPV16. 11 smears showed HPV18/45 (N=11 patients), 9 smears P4 (N=9 patients) and 9 smears P5 (N=8) types. In four patients,

anoscopy and cytology were negative while HPV was present. 3 smears showed ASCUS (Atypical Squamous Cells of Undetermined Significance) (5%), one showed ASCH (Atypical Squamous Cell evocating High grade lesion) (2%), 23 showed LSIL (39%) and 12 showed HSIL (20%). 17 smears showed no abnormality (29%). 3 smears were non contributive (5%).

195 cervical smears were obtained in 190 women (5 patients had 2 separate cervical smears). Median age was 45 years (23-79). Oncogenic-HPVs prevalence was 38% (N=72 patients).

P3 predominated in cervical smears (N= 36 women, 37 smears). 13 smears showed HPV16 (N=13 women), 16 smears showed HPVs18/45 (N=16 women), 15 smears P4 (N=15 women) and 22 smears P5 (N=21 women) types. Multiviral lesions were noted in 21 patients. 14 smears showed ASCUS (7%), 50 showed LSIL (26%) and 12 showed HSIL (6%). None showed ASCH. 117 smears showed no abnormality (60%). 2 smears were non contributive (1%).

Conclusion

In this HIV+ immunocompetent population under highly active anti-retroviral treatment, the prevalence of oncogenic HPV lesions remained at high level. Oncogenic-HPV prevalence was higher in anus (73%) than in cervix (38%). Rapid PCR (1 hour) linked to cytology eliminated non-HPV related ASCUS (inflammatory lesions). In four patients, anoscopy and cytology were negative while oncogenic-HPV was present allowing appropriate patient follow-up.

P12-19

DETECTION OF HIGH-RISK HPV GENOTYPES AMONG THE ECUADORIAN FEMALE POPULATION: STUDY OF 1643 CASES WITH ROCHE COBAS 4800 HPV

H. Zambrano¹, C. Morales¹, A. Lee², F. Plaza³, D. Vanden Broeck⁴

¹Laboratorio Genética Molecular Hospital Luis Vernaza (Ecuador), ²Geisel School of Medicine (United States of America), ³Ginecología Hospital Luis Vernaza (Ecuador), ⁴ICRH University of Gent (Belgium)

Background / Objectives

While it is widely known that HPV 16 and 18 are responsible for approximately 70% of cervical cancer cases worldwide, limited information exists about the HPV genotype distribution in Ecuador.

Our objective is to detect the prevalence of High Risk HPV genotypes (HR-HPV) in the general Ecuadorian female population attending a reference center

Methods

Samples were taken from patients at the gynecology department at Hospital Luis Vernaza, Guayaquil, Ecuador from May 2015 to November 2015. Cervical samples were collected with Rovers Cervex brushes and then placed in PreservCyt Solution. Samples were then run on the Roche Cobas 4800 according to the manufacturer's protocol. Roche Cobas 4800 can detect HPV 16, HPV 18 and -"other hr-HPV genotype," indicating the presence of a hrHPV genotype(s) other than HPV 16/18.

Results

Screening indicated the presence of an hr-HPV genotype in 266 of the 1643 total cervical samples (16.2%). Of these 266 samples, 246 only had one of the three markers positive. 35 samples were positive for HPV-16, 15 were positive for HPV-18 and 196 were positive for a-"other hr-HPV. Co-infections were reported in 20 cases. Co-infection of both HPV-16 and HPV 18 was found in one sample; co-infection of HPV 16 and another hr-HPV genotype, other than HPV 18, was present in 13 cases, whereas co-infection of HPV-18 and other hr-HPV was detected in 6 cases. No sample had all three of these markers positive. In total, we found 49 samples positive for HPV-16 and 21 samples positive for HPV-18.

Conclusion

This study provides new epidemiological data concerning the hr-HPV distribution in Ecuador. The distribution of hr-HPV infection is similar to those reported in other populations. Determining the prevalence of HPV 16 and HPV 18 is important since the current HPV vaccines available in Ecuador prevent the infections of those genotypes. Up until today, this is one of the largest studies using a clinically validated assay to determine the prevalence of hr-HPV genotypes in Ecuador.

P12-20

SOCIODEMOGRAPHIC, PRACTICAL AND ATTITUDINAL BARRIERS TO CERVICAL CANCER SCREENING IN UNDERSCREENED AND NEVER SCREENED JAPANESE WOMEN

S. Hanley¹, H. Fujita², H. Sato³, A. Nakamura³, N. Sakuragi¹

¹Hokkaido University Graduate School of Medicine, Sapporo (Japan), ²Cytology Center, Hokkaido Cancer Society, Sapporo, (Japan), ³Ebetsu Health Center, Department of Health and Welfare, Ebetsu (Japan)

Background / Objectives

Cervical cancer screening attendance in Japan has stagnated at around 30%. HPV self-sampling may help overcome some of the barriers to screening. However, few studies have investigated barriers to screening in under or never screened Japanese women. We investigated endorsed barriers to cervical cancer screening and compared barriers endorsed by women up-to-date with screening, to those under or never screened.

Methods

Anonymous self-administered questionnaires investigating sociodemographic, practical and attitudinal barriers to cervical cancer screening were sent to 473 women in Ebetsu city northern Japan, who were aged 20-44yrs and had requested an HPV self-sampling kit between October 2014 and January 2015. Logistic regression models were used to explore associations between socioeconomic factors, barriers and screening status. The study was approved by the IRB of Hokkaido University Graduate School of Medicine and Hokkaido Cancer Society

Results

In total 392 (82.9%) women returned the self-sampling kit and questionnaire, and 389 were used in the final analysis. Eighty-seven (22.4%) of women were up-to-date for screening, while 135 (35%) and 166 (42.7%) were underscreened and never screened, respectively. While 'Difficult to get an appointment due to work or childcare' (75.3%), and 'Pap smears are embarrassing' (67.4%) were commonly endorsed barriers, they were not significant predictors of screening attendance. 'I intend to go, but don't get round to it' (OR 3.91, 95% CI 1.61-9.53); 'I don't feel at risk for cervical cancer' (OR 3.71, 95% CI 1.34-10.27); and being a current smoker (OR 3.15, 95% CI 1.27-7.78) were predictors of being underscreened. For never screened, 'I worry the Pap smear will be painful' (OR

2.11, 95% CI 1.18-3.80); 'I don't feel at risk for cervical cancer' (OR 3.47, 95% CI 1.19-10.06); being aged 20-24yrs (OR 0.17, 95% CI 0.04-0.81); being single (OR 0.80, 95% CI 0.29-2.22) and being a current smoker (OR 4.80, 95% CI 1.93-12.00) were significant.

Conclusion

While HPV self-sampling may be helpful to reduce some of the barriers associated with cervical screening in Japanese women, more education about actual risk factors for cervical cancer is also essential.

P12-21

COMPATIBILITY OF GENEFIRST PAPILOPLEX™ HR-HPV GENOTYPING ASSAY FOR TESTING FIRST VOID URINE SPECIMENS

A. Vorsters¹, V. Vankerckhoven², D. Kapadia³, M. De Koeijer², M. Moreau³, G. Fu³, C. Proudlove³, K. Beyers², S. Biesmans¹, S. Van Keer¹, P. Van Damme¹

¹University of Antwerp, VAXINFECTIO (Belgium), ²Novosanis (Belgium),
³GeneFirst (United kingdom)

Background / Objectives

Background: HPV urine testing has been proposed for monitoring impact of HPV vaccination, follow-up of treatment and/or reaching women not participating in cervical cancer screening programmes. The use of Colli-Pee™ (Novosanis) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine. The Genefirst Papilloplex™ HR-HPV assay can genotype and quantify all 14 high risk types in a single closed tube real-time PCR reaction. The assay has been previously evaluated on liquid based cytology (LBC) cervical screening samples but not on urine samples for HPV detection.

Objectives: The aim of this study was to determine if Genefirst Papilloplex™ HR-HPV test is compatible with self-collected first void urine specimens. We compared the results of the Papilloplex™ HR -HPV test with results of the in-house real time type specific PCR used at AML and the Optiplex HPV genotyping Kit from DiaMex.

Methods

Methods: Women who had a self-reported prior HPV positive test result were enrolled in the study. 172 first void urine samples, provided by 22 women, were collected using either the Colli-Pee™ (Novosanis), first void urine collection device (n=86), or directly into a urine cup (n=86). The participants had to alternate the collection times (morning and late afternoon) over 4 consecutive days. Prior to the PCR tests four ml of urine/UCM mixture was concentrated on an ultrafiltration membrane and extracted with easyMag® (bioMérieux).

Results

Results: There was substantial agreement for high risk HPV DNA positivity between the Genefirst results and the AML and DiaMex results. Respectively for AML/Genefirst and DiaMex/Genefirst an agreement of 86%; kappa 0.688 and agreement of 87.7%; kappa 0.705 was observed. The quantitative results are being analysed and will be presented at the meeting.

Conclusion

Conclusions: These preliminary results confirm that the Papilloplex™ HR-HPV assay is compatible with self-collected first void urine. Clinical cut-off determination will be addressed in future studies

P12-22

NON-INVASIVE FIRST-VOID URINE SAMPLING AND ACCEPTABILITY TO MONITOR THE IMPACT OF VACCINATION AGAINST HPV

S. Van Keer¹, A. Vorsters¹, S. Biesmans¹, X. Van Ostade², T. Wiebren³, M. Ieven¹, P. Van Damme¹

¹Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp (Belgium), ²Proteinchemistry, proteomics and epigenetic signaling (PPES), University of Antwerp (Belgium), ³Gynaecological cancer, Antwerp University Hospital (Belgium)

Background / Objectives

Feasibility of first-void urine (FVU) sampling to monitor the impact of HPV vaccination has recently been reported [1]. As limited data are available on sampling preference in vaccinees, we assessed the acceptance of using FVU self-sampling as a monitoring tool in vaccinated and unvaccinated women.

Methods

At the University of Antwerp, 56 FVU samples were collected (Colli-Pee™, Novosanis) from 19 to 26 year old women (mean: 22.30 ± 2.01 year), from whom 19 were unvaccinated, and 37 were previously vaccinated with the bi- or quadrivalent prophylactic HPV vaccine (NCT02714114). Data regarding acceptability of FVU sampling were gathered through questionnaires. HPV DNA genotyping was performed with the Optiplex HPV genotyping assay (Diamex GmbH) after Amicon filtration (Merck Millipore) and NucliSENS® easyMAG® DNA extraction (bioMérieux) [2]. Statistical analysis was performed using IBM Statistics SPSS software Version 23.

Results

From women who previously used a standard urine cup, had a pap smear taken by a clinician, or had a blood sample taken, respectively 46/50; 28/31; and 42/55 preferred FVU sampling with the Colli-Pee™ device over the other method.

An overall prevalence of HPV types 16/18 of 13% (7/56) was found, with a lower percentage in vaccinated versus unvaccinated subjects, respectively 8% (3/37) and 21% (4/19) (OR: 3.022 (95% CI 0.601-15.203) for being HPV16/18 negative (vaccinated/unvaccinated)). No HPV6/11 was found.

The mean age of first sexual contact in the vaccinated and unvaccinated cohort was respectively 17.54 ± 1.85 and 16.40 ± 1.55 year ($p < 0.05$; unpaired student T-test, equal variances assumed), and the mean age of vaccination was 15.79 ± 1.70 year. Two out of 37 vaccinated, and 4/19 unvaccinated women were not sexually active yet, and all HPV DNA negative. From the 35/37 vaccinated women reporting having sexual contact before, 26/37 were vaccinated before first sexual contact, from whom 3/26 tested HPV16 positive, whereas none of the HPV vaccine types were detected in 9/37 vaccinated women reporting to be sexually active before vaccination. In the unvaccinated cohort reporting having sexual contact before (15/19), 3/15 tested positive for HPV16 and 1/15 for HPV18.

Conclusion

Consistent with previous studies, our study illustrates the acceptance of FVU sampling in this population. In addition, feasibility of FVU to detect HPV DNA in vaccinees was shown. The HPV DNA positives seen in this cohort can partly be explained by the highly-sensitive multiplex assay used, and the “non-sexual” exposure to HPV before start of vaccination [1]. The latter stresses the need of vaccination against HPV in pre-adolescent stage.

References

1. Franceschi S, Umulisa MC, Tshomo U, Gheit T, Baussano I, Tenet V, et al. Urine testing to monitor the impact of HPV vaccination in Bhutan and Rwanda. *Int J Cancer* 2016.
2. Vorsters A, Van den Bergh J, Micalessi I, Biesmans S, Bogers J, Hens A, et al. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. *Eur J Clin Microbiol Infect Dis* 2014,33:2005-2014.

P12-23

DETECTION OF HIGH GRADE CIN USING HPV DNA TESTING WITH PARTIAL GENOTYPING FOR HPV16/18

S. Kikawa¹, H. Fujita², S.J.B. Hanley³, T. Akiko³, K. Mitsunori², S. Takayuki², S. Tsuyoshi⁴, S. Kazuo⁵, N. Sakuragi³

¹Otaru General Hospital (Japan), ²Hokkaido Cancer Society (Japan), ³Hokkaido University Graduate School of Medicine (Japan), ⁴Sapporo Medical University (Japan), ⁵Asahikawa Medical University (Japan)

Background / Objectives

High risk HPV (hr-HPV) testing is being widely incorporated in cervical screening globally. Japan still recommends biennial Pap smears as the standard screening method. We conducted a 3yr prospective study to evaluate the usefulness of cytology and HPV testing (cotesting) with partial genotyping for HPV16/18. Here baseline data is presented.

Methods

Women aged 20-69yrs attending Hokkaido Cancer Society for cervical screening in 2014 were informed about the study. Consenting participants had a Pap test and an HPV test using the Cobas 4800 System (Roche Diagnostics) which detects HPV16, HPV18, and 12 other hr-HPVs. Age-specific hr-HPV prevalence and HPV 16/18 prevalence at 1st screening visit as well as detection rates of CIN2+ lesions in relation to HPV/cytology status within 12mth of the 1st visit were investigated. This study was approved by the IRB for clinical trials at Hokkaido University.

Results

In total, 14,650 women were enrolled. The mean age of participants was 50.6yrs. Hr-HPV detection rates for women in their 20s, 30s, 40s, 50s, and 60s was 16.1%, 8.8%, 5.2%, 2.6% and 2.7%, respectively. Of these 36.6%, 32.9%, 18.4%, 16.8% and 24.6% were HPV16/18 positive. Abnormal cytology was found in 347 women (2.4%). By age group it was 8.2%, 4.4%, 3.4%, 1.4% and 0.7%, respectively. Detection of HPV 16/18 was significantly higher in CIN2+ cases compared to other hr-HPV types (Odds ratio 3.80, 95% Confidence Interval 2.4-5.9). Incidence of CIN2+ in women with NILM cytology was 13/99 women (13.1%) with HPV16/18 and 5 /353 women (1.4%) with other hr-HPV ($p < 0.001$). One woman who was HPV18 positive with NILM cytology and an initial negative colposcopy, presented with adenocarcinoma at the 2nd colposcopy 5 months later.

Conclusion

Women HPV16/18 positive, even with negative cytology, are at a high risk for the development CIN2+ lesions within 12 months and should be recommended for colposcopy and directed biopsy with intensive follow-up. Prevalence of HPV 16/18 is highest in women in their 20s/30s and these women have an equally high-risk of CIN2+ lesions as women in their 40s/ 50s.

P12-24

HPV TESTING FOR CERVICAL CANCER SCREENING: EXPERIENCE IN CENTRO MEDICINA LABORATORIAL GERMANO DE SOUSA/HOSPITAL CUF DESCOBERTAS

M. Sousa, R. Ribeiro

Centro Medicina Laboratorial Germano de Sousa (Portugal)

Background / Objectives

Human Papillomavirus (HPV) is a well-studied etiologic agent for cervical cancer. The HPV test, as primary method of cervical cancer screening, decreases the incidence of invasive carcinoma, and its performance is superior when compared to cytology.

The aim of this study was emphasizing the overall performance of the methods used by CML-GS and correlate the results with cytological examinations in a sample population from HCD.

Methods

From January-2012 to Decemeber-2015 were analyzed 4685 cervical samples by HPV-molecular and conventional-cytology methods. HPV-molecular methods used where: Hybrid-Capture2; Cobas-HPV test; Clart Human papillomavirus 2; PapilloCheck.

The cytological results were registered with SNOMED nomenclature: Normal; ASC-US; ASC-H; Glandular cell atypia; LIEBG; LIAG and Squamous cell carcinoma

Results

Hybrid-Capture2:

1. 425 (26.02%) as HPV-HR positive and 1208 (73.97%) as HPV-HR negative.
2. detection-rate 54.92%; 83.88% specificity; 12.00% FPR; false-negative-rate of 11.51%.

Cobas-HPV test:

1. 631 (22.19%) as HPV HR positive and 2213 (77.81%) as HPV HR negative.
2. detection-rate 59.12%; 84.85% specificity; 12.73% FPR; false-negative-rate of 6.54%.

Genotyping-HPV test:

1. 105 (47.51%) as HPV HR positive and 116 (52.49%) as HPV HR negative.
2. detection-rate 69.14%; 65.00% specificity; 22.17% FPR; false-negative-rate of 11.31%.

Most frequent HR-types: HPV53(11.98%), HPV16(10.78%), HPV66(10.18%), HPV51(7.78%). Multiple HPV-infections decreased by the cytological severity.

Conclusion

Our HPV type prevalence findings were similar from others found in populations HPV studies. The most frequent hr-HPV in Portuguese population is HPV53, where the malignancy rate is not as high as 16/18. We can expect a HPV type shift, possibility from universal vaccination.

The hc2 and 16/18-Cobas accomplished concordance in false-positive-rate, detection-rate and specificity. The statistically significant differences (p -value <0.05) are 16/18-Cobas yield lower false-negative-rate for Abnormal Cytological results, subsequently higher negative-predictive-value, induce 16/18-Cobas testing better for triage.

P12-25

XPert HPV ASSAY AS A PROMISING TEST IN CLINICAL SETTING: ANALYTIC AND DIAGNOSTIC PERFORMANCES.

R. Zappacosta¹, F. Sablone¹, F. Di Toro¹, L. Pansa², S. Rosini¹

¹Department of medical, oral sciences and biotechnology, G d'Annunzio University of Chieti-Pescara (Italy), ²School of Biochemistry, G d'Annunzio University of Chieti-Pescara (Italy)

Background / Objectives

Evidence-based guidelines strongly recommend HPV testing in primary screening to prevent cervical cancer. Many HPV test are currently available but four were FDA approved: Hybrid Capture 2 (HC2, Qiagen), Cervista (Hologic), Cobas HPV (Roche), and Aptima (Gen-Probe). These technologies, requiring laboratory expertise and sophisticated platforms, are often time consuming and demonstrated poor feasibility in routinely clinical settings. Xpert HPV assay (Cepheid) is a new, rapid, qualitative Real-time PCR assay, able to detect E6/E7 genes of 14 oncogenic HPV types. DNA extraction, amplification and target detection take place in a disposable cartridge, complete of all reagents (1). The test requires only one hour to be completed. The first goal of this retrospective study was to investigate the analytic performances of Xpert HPV test on up to 11-year-old residual liquid-based cervical samples. Then, in accordance with Meijer's criteria (2), we assessed the accuracy of Expert HPV. Comparisons with EasyQ HPV mRNA test (Biomerieux) and HC2 test were also done.

Methods

In our institution is placed a Tissue and Cell Biobank. Here, starting from 2001, residual PreservCyt cervical specimens are archived. We retrieved 98 specimens, collected during the years 2004-2015, and related to women referred to colposcopy for abnormal cytology result. The inclusion criteria were: HC2 and RNA test results, histological diagnosis as goal standard. Xpert HPV was performed on each single sample.

Results

Xpert HPV test showed 6% of invalid results. A weak correlation between invalid test results and age of specimens has been found ($r=0.12$). At CIN2+ threshold, sensitivity and specificity were 90 and 23.5%, respectively. The unique cancer case resulted as positive. Percentage of positive samples

increased with the severity of histological diagnosis. Concordance with HC2 ($k=0.67$), and mRNA test ($k=0.61$) were substantial.

Conclusion

These preliminary data demonstrated the excellent analytic performances of Xpert HPV DNA in residual up-to 11-year old liquid-based samples. Sensitivity appears to be equivalent to those of approved HPV tests. Specificity was low, probably due to characteristics of study population. This fact would emphasize the need of adjunctive, more specific triage test. The good agreement between Expert and E6/E7 mRNA test (both including the five most oncogenic HPV types) would support the use of E6/E7 sequences to bypass false negative results subsequent to the use of target L1 sequence (high-grade lesions are usually associated with HPV integration and loss of L1 gene). Finally, Xpert HPV assay offers simplicity of execution, rapid turnaround time and suitability for clinical setting.

References

1. Cuzich J et al. Performance of Xpert HPV assay in women attending for cervical screening. *Papillomavirus Research* 2015.
2. Meijer CJ et al. Validation of high-risk HPV test for primary screening. *J Clin Virol*, 2009

P13-01

COMPARATIVE STUDY OF THE IMMUNOMODULATORY PROPERTIES OF DIFFERENT CORIOLUS VERSICOLOR-BASED FORMULATIONS ADMINISTERED ORALLY OR TOPICALLY IN THE VAGINA IN HEALTHY MICE.

J. Gálvez¹, T. Vezza¹, J. Garrido-Mesa¹, A. Rodríguez-Nogales¹, F. Algieri¹, D. Camuesco¹, M.E. Rodríguez-Cabezas¹, J. Cortés², D. Dexeus³

¹CIBER-EHD, Department of Pharmacology, ibs.GRANADA, Center for Biomedical Research (CIBM), University of Granada, Granada, (Spain), ²Senior Consultant on Gynecologic Oncology. Former President of the Spanish Association of Colposcopy and Cervical Pathology, Palma de Mallorca, (Spain), ³Gynecology and Obstetrics Department, SOMDEX-Clínica Tres Torres, Barcelona, (Spain)

Background / Objectives

Preclinical study to compare the modulation of the immune response of vaginal administration of a Coriolus versicolor-based gel formulation (Palomacare®) in healthy mice with another gel containing only 5% Coriolus versicolor (CV5%) and Coriolus versicolor extract (CVext) administered orally.

Methods

Female CBA/J (J-2 α) mice (8 to 10 weeks of age) were daily inoculated in the vagina with 50 μ l of Palomacare® (L-1505) or CV5% (P-7449), or orally administered a CVext (500 mg/kg) (n=14). A control group (n=8) was inoculated with saline solution. Half of the mice were sacrificed at day 6, and the rest at day 10. The vagina was excised, weighed and two samples were obtained. One sample was stored in RNAlater and processed for RNA extraction and the expression of different cytokines (TNF α , IL-1 α , IL-1 β , IL-6, IL-17, IL-13 and IFN- α) was evaluated by real time RT-qPCR. The other tissue sample (2 mg) was cultured for 24 h at 37°C in a 5% CO₂ atmosphere; then, the culture media was collected, centrifuged and stored at -80°C. Cytokine analysis could be performed in this sample.

Results

IFN- α and IL-13 were not modified by any treatment (the expression of IL-13 was under the limit of detection). At day 10 we observed that all the treatments significantly up-regulated the gene expression of most of the other cytokines tested ($p < 0.05$), except for IL-6 that was only increased by CV5% gel. No significant differences were observed when the treated groups were compared. At day 6, Palomacare[®] already managed to increase the expression of most of the cytokines tested except for IL-6 and IL-17. CV5% gel only increased TNF α and IL-1 α expression, whereas oral treatment with CVext did not induce any cytokine modifications.

Conclusion

Palomacare[®] gel managed to activate the vaginal immune response faster than the oral administration of CVext. Both preparations (oral and vaginal) have demonstrated to equally increase significantly the immune response vs control group at 10 days. Additional experiments should be conducted to further investigate their potential use in the prevention and/or treatment of genital tract infections.

P14-01

PRECANCEROUS AND CANCEROUS LESIONS OF THE UTERINE CERVIX IN KINSHASA: ASSOCIATION WITH HIV INFECTION

C. Ali-Risasi¹, K. Verdonck², D. Vanden Broeck³, M. Praet⁴

¹Laboratory of Anatomopathology, General Reference Hospital of Kinshasa (Congo, the democratic republic of the), ²Institute of Tropical Medicine Antwerp (Belgium), ³International Centre for Reproductive Health (ICRH), Ghent University Hospital (Belgium), ⁴Department of Medical and Forensic Pathology, Ghent University Hospital (Belgium)

Background / Objectives

Cervical cancer is frequent in Sub-Saharan Africa. Moreover, as the access to antiretroviral treatment improves, it is expected that chronic HIV-related diseases such as cervical cancer will increasingly come to the foreground. In the Democratic Republic of the Congo, little information is available about the frequency of cervical cancer in women with and without HIV. The study aim was to estimate the strength of the association between HIV infection and the presence of (pre)cancerous lesions of the uterine cervix in women from Kinshasa.

Methods

We conducted a cross-sectional study and enrolled HIV-positive and HIV-negative women in two HIV screening and treatment centres in Kinshasa in 2006-2007. Cervical smear samples were examined using liquid-based cytology and classified according to the Bethesda 2001 classification of cervical pathology. Women with cytology results indicating low-grade squamous intraepithelial lesions or higher-grade lesions (LSIL+) were considered to have (pre)cancerous lesions. We collected information about sociodemographic and behaviour-related factors that could act as confounders in the relation between HIV infection and LSIL+, i.e. age, marital status, socio-economic status, smoking, age at first sexual intercourse, number of lifetime sex partners, childbirths and habit of vaginal cleansing. Multiple logistic regression was used to check for confounding and effect modification and to calculate an adjusted odds ratio (OR) for the association between HIV and LSIL+. In addition, among HIV-positive women, we described the relation between CD4 cell counts and LSIL+ and used the Mann-Whitney *U* test to compare groups.

Results

One hundred twenty-eight HIV-positive and 132 HIV-negative women were included. Their mean age was 34 years (standard deviation 10). Five HIV-negative (4%) and 41 HIV-positive women (31%) were diagnosed with LSIL+. The final logistic regression model included five variables: HIV, number of childbirths, marital status, number of lifetime sex partners, and age at first sexual intercourse. HIV was the only variable for which the association with LSIL+ remained significant on multivariable analysis. The adjusted OR for the association between HIV infection and presence of LSIL+ was 7.5 (95% confidence interval 2.6-21.4). CD4 cell counts were available for 87 HIV-infected women: those with LSIL+ had lower CD4 counts (median 162 cells/ μ l; interquartile range 93-327) than those without LSIL+ (336 cells/ μ l; 184-475; $P=0.002$).

Conclusion

Among women of Kinshasa, there is a strong association between HIV infection and the presence of precancerous or cancerous lesions of the uterine cervix.

P15-01

INVESTIGATION OF MEMBRANE PROTEINS OFFERS SIGNIFICANT INSIGHTS ON THE PROCESS OF CERVICAL CANCEROGENESIS

P. Christou¹, G. Mermelekas¹, V. Lygirou¹, G. Kontostathi¹, M. Makridakis¹, J. Zoidakis¹, A. Vlahou¹, N.P. Anagnou², K.I. Pappa³

¹Biotechnology Laboratory, Centre of Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece (Greece),

²Laboratory of Biology, University of Athens School of Medicine, Athens,

Greece (Greece), ³First Department of Obstetrics and Gynecology, University of Athens School of Medicine, Athens, Greece (Greece)

Background / Objectives

Cervical cancer is the third most common malignancy in women worldwide. Membrane proteins are involved in cell signaling, cell-cell interactions and transportation. Despite the critical biological significance of membrane proteins, proteomic analysis has been a challenging task, due to their particular biochemical properties. Furthermore, dysfunction of membrane proteins has been shown to correlate with the malignant phenotype in several cancers. Therefore, their systematic study could lead eventually to the discovery of novel drug targets and biomarkers for prognostic or diagnostic purposes. The aim of this study was to compare the expression pattern of membrane proteins of one normal (HCK1T) and three cervical cancer cell lines, i.e. C33A (HPV-), SiHa (HPV16+) and HeLa (HPV18+), in order to discover proteins which may constitute potential biomarkers for cervical cancer.

Methods

The procedure used for membrane fraction preparation, involved differential centrifugation and detergent-based solubilization, followed by trypsinization. Peptides were fractionated and identified by high resolution LC-MS/MS. Differentially expressed proteins in cancer cell lines relative to HCK1T, exhibited >2 or <0.5 fold change with a $p < 0.05$ (Mann-Whitney test).

Results

An efficient and reproducible enrichment protocol for membrane proteins was developed. The percentage of membrane proteins in membrane extracts was within the range of 38.0%-42.5% and the percentage of transmembrane proteins within a range of 20.2%- 24.3%. These percentages were

significantly higher compared to the total cell extracts. A significant number of unique membrane and transmembrane proteins were identified in the membrane extracts. The average fold enrichment for membrane proteins compared to the total cell extracts was 1.46 and for transmembrane proteins 2.21. The proteomic analysis revealed a variety of differentially expressed membrane proteins involved in signaling pathways associated to cancerogenesis. The identified proteins included Vesicle transport protein GOT1B (testicular seminomas and ovarian carcinoma), GTP-binding protein SAR1b (liver cancer) and ATP-dependent RNA helicase DDX3X (hepatocellular carcinoma).

Conclusion

Unique membrane protein identifications can offer insights on a previously inaccessible part of the cell proteome. Therefore, the successful isolation of membrane proteins in this study generated a significant pool of potential cervical cancer biomarkers and novel promising drug targets. These can be functionally tested in vitro utilizing gene editing approaches, such as the CRISPR/Cas9 system.

P15-02

NEW SCREENING PROTOCOL WITH DUAL-STAINED CYTOLOGY TRIAGE

M.D. Comes, R. Oncins, M.Á. Aragón, E. Clemente, V. Vallés, G. Muñiz, L. Guardia

Hospital de Barbastro (Spain)

Background / Objectives

Objectives: the objective of this study was to identify CIN2+ patients with p16/ki67 dual-stained cytology in patients with ASCUS/LSIL cytology result. To confirm the CINtec PLUS® usefulness.

Methods

Methods: 2092 gynecology cytologies were performed between March 1st 2015 and December 31st 2015, resulting in 157 ASCUS/LSIL cytologies. Dual-stained was performed in 75 out of 157 patients. Dual- immunostaining for p16/ki67 was performed using the CINtec PLUS® kit (Roche mtm laboratories, Mannheim, Germany). CINtec PLUS® was used on a slide processed with Thinprep 2000® (Hologic, Bedford, MA, USA) with the residual material of Preservcyt liquid cytology. In conventional cytology cases, dual-stained was realised with the HPV testing residual material. We studied 75 CINtec PLUS® cases that belong to 73 patients. Negative patients are controlled in 12 months with a new HPV test. Positive dual-staining patients were referred to colposcopy and biopsies were taken if clinically indicated. Most of the studied patients had an HPV test performed by a polymerase chain reaction in the Cobas 4800® system including 3 option results, HPV-HR +/- for 16 genotype, HPV-HR +/- for 18 genotype and HPV-HR +/- for a pool of 12 other genotypes.

Results

Results: Patients average age was 39.85 years (SD 11.78). ASCUS/LSIL prevalence was 7.5%. 31(41.4%) out of 75 dual-stained cytologies were positive and 43(57.3%) were negative. There was one (1.3%) unsatisfactory case. Negative CINtec PLUS® cases are equal to avoid colposcopies. About dual-staining cytologies, 34 cases were LSIL and 41 ASCUS. Of the total ASCUS cases, 27(65.9%) were CINtec PLUS negative and 14(34.1) were positive. About LSIL cases, 16(47.1%) were negative and 17(50%) positive, with one case (2.9%) having unsatisfactory dual-staining. Of the 31 CINtec PLUS® positive cases there were biopsies performed in 19 cases. This biopsy results were 7 negative cases, 11 CIN1 and 2 CIN3 (one coming from ASCUS and the other one from LSIL). In the other 12 cases the biopsy wasn't performed.

Conclusion

Conclusions: Dual-staining adds efficacy to the triage with cytology due to the fact that almost two thirds of the ASCUS are CINtec Plus® negative. These cases can be followed up in one year with security. Dual-staining avoid unnecessary colposcopies especially in ASCUS patients. The decrease of colposcopies avoid anxiety and inconvenience to the patient, decreases the healthcare network pressure in cervical pathology consults and decreases the screening expense. Despite of being a reduced series, this study has shown the usefulness of dual-stain in as much ASCUS as in the LSIL lesions.

References

1. Ikenberg H, Bergeron C, Schmidt D, et al. Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: results of the PALMS study. *J Natl Cancer Inst* 2013; 105:1550–7.
2. Schmidt D, Bergeron C, Denton KJ, et al. p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou cytology: results from the European equivocal or mildly abnormal Papanicolaou cytology study. *Cancer Cytopathol* 2011;119:158–66
3. Petry KU, Schmidt D, Scherbring S, et al. Triage Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. *Gynecol Oncol* 2011; 121:505–9.
4. Uijterwaal MH, Witte BI, Van Kemenade FJ, et al. Triage borderline/mild dyskaryotic Pap cytology with p16/Ki-67 dual-stained cytology testing: cross-sectional and longitudinal outcome study. *Br J Cancer* 2014;110:1579–86

P15-03

PERFORMANCE OF THE ONCOE6 TEST FOR HIGH- GRADE CERVICAL DISEASE AND CANCER DETECTION AMONG HONDURAN WOMEN

A. Ferrera¹, W. Valladares¹, Y. Cabrera¹, M.L. Hernandez², R. Herrero², M. Almonte²

¹Escuela de Microbiología, Facultad de Ciencias, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras (Honduras), ²Prevention and Implementation Group, International Agency for Research on Cancer, World Health Organization, Lyon, France (France)

Background / Objectives

Cervical cancer is a public health problem in Honduras. Cytology-based screening has failed in reducing cervical cancer rates in the country, and moving towards HPV primary screening is under evaluation. The ESTAMPA study aims to evaluate triage techniques for HPV positive women aged 30-64 years within an HPV-based screening programme. One of these techniques is the OncoE6 test, a qualitative test that detects levels of E6 oncoprotein expressed by HPV types 16 and 18. The test is robust, simple, fast (2.5 hours) and not very costly making it very promising for low-resource settings. We evaluate the performance of the OncoE6 test using samples from women in the ESTAMPA study, supplemented with samples from a referral population.

Methods

Cervical samples were collected from two different groups of women: 155 attending HPV-screening in the ESTAMPA study (screening group) and 41 attending colposcopy after HSIL cytology (referral group). Samples were tested blindly with Hybrid Capture II (hc2), the LiPA (Genotyping kit HPV GP, version 2, primers GP5+/GP6+) for genotyping and the OncoE6 test. HPV positive women in the ESTAMPA group and all the HSIL's underwent colposcopy and biopsies were collected from visualised lesions and interpreted by a local pathologist. Estimates of sensitivity and specificity with corresponding 95% confidence intervals (95CI) for detection of cervical intraepithelial neoplasia grade 2 or worse lesions (CIN2+) of the OncoE6 test were obtained.

Results

Ninety five women tested positive for HPV: 93 in both hc2 and LiPA and two only in LiPA. Twenty-one women tested positive for HPV16 and nine for HPV18 oncoproteins using the OncoE6 test. A total of

seven CIN2's, 6 CIN3's and 31 cancers were diagnosed. Two CIN2's and one cancer were detected in women in the screening group; all other cases were diagnosed in the referral group. The OncoE6 test was positive in all CIN2+ associated to HPV16/18 (three CIN2, four CIN3 and 19 cancers), in one cancer that tested negative in all other tests, and in three women with no high-grade disease. The sensitivity of the OncoE6 was 61.4% (95CI:45.5%-75.6%) for detection of CIN2+ and 100% (94.3-100) for CIN2+ associated to HPV 16 or HPV 18; the specificity for less than CIN2 was 98% (95CI:94.3%-99.6%).

Conclusion

The OncoE6 test showed high concordance with the LiPA genotyping, detected 61% of all precancer and cancers, and 100% of cases associated to HPV16/18. In addition, it showed very high specificity highlighting its potential to be used to triage HPV positive women.

P15-04

SYSTEMS BIOLOGY STUDY FOCUSING ON THE SECRETOME OF CERVICAL CELL LINES REVEALS MATRIX REMODELING AND OXIDATIVE STRESS RESPONSE AS PIVOTAL PATHWAYS IMPLICATED IN CARCINOGENESIS

G. Kontostathi¹, **M. Makridakis**¹, **V. Lygirou**¹, **A. Vlahou**¹, **J. Zoidakis**¹, **N. Anagnou**², **K. Pappa**³

¹Biotechnology Laboratory, Centre of Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece (Greece),

²Laboratory of Biology, University of Athens School of Medicine, Athens, Greece (Greece),

³First Department of Obstetrics and Gynecology, University of Athens School of Medicine, Athens, Greece (Greece)

Background / Objectives

Cervical cancer results from infection by certain HPV types. Specifically, HPV types 16 and 18 are responsible for 70% of cervical cancers and precancerous cervical lesions. The aim of the study was to identify proteins and biological processes relevant to cervical malignancy by proteomic analysis of the secretome from normal and cancer cell lines.

Methods

Secreted proteins were collected from three representative cervical cancer cell lines, SiHa (HPV16+), HeLa (HPV18+), C33A (HPV-) and HCK1T a normal cervical epithelium cell line which served as control. The secretome samples were analyzed by high resolution mass spectrometry LC/MS-MS and evaluated by Proteome Discoverer 1.4, while only high peptide confidence identifications were accepted. The proteins with statistically significant (Mann Whitney $p < 0.05$) differential expression (fold change from >2 to <0.5), were subjected to bioinformatics analysis by the Ingenuity Pathway Analysis (IPA) software.

Results

The LC/MS-MS analysis identified 1200-1300 secreted proteins for each cell line, a significantly higher number compared to the 300-400 proteins identified by 2D electrophoresis coupled to MS. A number of proteins with important biological functions were identified, such as the extracellular matrix remodeling proteases Cathepsins, which were upregulated in cancer cell lines (SiHa, HeLa, C33A) vs

normal (HCK1T). Bioinformatic analysis revealed significant pathways deregulated in cervical cancer such as Inhibition of Matrix Metalloproteases (MMPs) and oxidative stress response. The proteomics results and bioinformatic predictions were validated in cervical cell lines and in clinical samples. High levels of Cathepsin D, a secreted protease that plays a pivotal role in remodeling and degradation of extracellular matrix, were detected in cancer samples by Western blot. Moreover, a cervical cancer-associated increase in MMPs enzymatic activity was determined by zymography assays. The under-expression of anti-oxidant enzyme Superoxide Dismutase 2 (SOD2) in cervical malignancy was confirmed by Western blot.

Conclusion

The systems biology approach used to study cervical cancer resulted in the discovery and subsequent validation of significant proteins and pathways that play important roles in malignancy. The most prominent findings included: proteases secreted by cancer cells, such as Cathepsin D and MMPs, which are involved in the process of invasion of the surrounding tissues, and enzymes implicated in oxidative stress response, such as SOD2.

P15-05

EXPRESSION OF p16/Ki67 DUAL-STAINING DEPENDING ON MOST COMMON HPV GENOTYPES IN CERVICAL LESIONS

I. Krivak Bolanca¹, S. Findri Gustek², V. Orescanin³

¹Unit for gynaecologic cytology, Department of Clinical Cytology and Cytogenetics, "Merkur" University Hospital, Zagreb (Croatia), ²Centre for gynecology, urology and occupational medicine, Sesvete, (Croatia), ³Orescanin Ltd., A. Jaksica 30, Zagreb, (Croatia)

Background / Objectives

Objective: The vast majority of HPV infections are cleared spontaneously, without treatment. If the viral infection persists, the risk of developing a precancerous lesion increases as well as the risk of developing an invasive carcinoma. HPV genotyping is of clinical interest, since the risk of developing a precancerous lesion varies depending on genotype. A number of biomarkers that allow monitoring essential molecular events are likely to improve the detection of lesions that have a higher risk of progression as well as predict progression of the cervical lesion. Aim of the study is to explore the expression pattern of p16/Ki-67 immunocytochemical dual-staining depending on specific, most common oncogenic human papilloma virus (HPV) genotype in cytological diagnosed low-grade lesion (LSIL) and high-grade lesion (HSIL) on cervical smears.

Methods

Material and Methods: One hundred forty-six patients with HPV testing results were selected from accompanied gynaecological practice. All these patients underwent cytological diagnosis and from additional smear, immunostaining was performed using CINTecPlus Kit (Roche)

Results

Results: Among 121 HPV positive patients, dual staining was positive in 69 (57%) patients, and 52 (42,9 %) of them had negative immunostaining. In group of 25 HPV negative patients, two of them had positive staining results. The overall prevalence of DNA HPV detection was 82,8% (121/146). Most frequent genotype was in the group of one or more other HPV types, then type 16, infection with multiple types which includes type 16, than type 18 in 52,1%, 28,1%, 14,8% and 4,9% respectively. Among 92 of patients with LSIL, positive p16/Ki67 staining was found in 46,7% (43/92) cases and was most frequent in patients with HPV infection type 16, one or more other types,

multiple infections including type 16 and type 18 in 17,4%, 14,1%, 11,9% and 3,2% respectively. Twenty-six (89,7%) of twenty-nine patients with HSIL diagnosis had positive dual staining in group of other oncogenic types in 44,8%; with type16 in 27,6%; in the group of multiple type infection 13,8% and one patient with HSIL, had infection with type18 (3,4%).

Conclusion

Conclusion: These results represent a strong association between positivity for oncogenic types of HPV, p16/Ki-67 staining and severe cytological abnormalities proving ones again importance of detecting oncogenic types in protocol of handling patients with cervical pathology. This methodology could be used to detect unnoticed cervical lesions. The identification of prognostic biomarkers that can predict progression to invasive cancers is an important, but challenging area of biomarker research.

References

Hwang J.S.,Shroyer K.R. Biomarkers of cervical Dysplasia and Carcinoma, *Journal of Oncology*; 2012;doi:10.1155/2012/507286

Doorbar J.: papillomavirus life cycle organization and biomarker selection. *Disease Markers*, 2007;23(4):297-303.

Nakamura Y.,Matsumoto K, Satoh T, Nishide K et al. :HPV genotyping for triage of women with abnormal cervical cancer screening results: a multicentre prospective study. *Int J Clin Oncol*.2015; 20(5): 974-81.

Killeen J.L. ,Dye T., Grace C., Hiraoka M.: Improved abnormal Pap smear triage using cervical cancer biomarkers. *J Low Genit Tract Dis*. 2014; 18(1):1-7.

WaldstrOm M., Christensen R.K.,Ornskov D.: Evaluation of p16/Ki67 dual stain in comparisom with an mRNA human papillomavirus test on liquid-based cytology samples with low-grade squamous intraepithelial lesion. *Cancer Cytopathology* 2013;121:136-45

P16-01

DETECTION AND QUANTIFICATION OF HPV-mRNA IN SENTINEL LYMPH NODES OF CERVICAL CANCER PATIENTS BY DIGITAL PCR AND REAL-TIME PCR – A COMPARISON OF METHODS

K. Carow¹, C. Read², N. Häfner¹, M. Dürst¹, A. Corner²

¹Department of Gynaecology, Jena University Hospital – Friedrich Schiller University Jena, 07743 Jena (Germany), ²RainDance Technologies, Billerica, MA 01821 (United States of America)

Background / Objectives

We recently showed that the presence of HPV-mRNA in sentinel lymph nodes (SLN) of cervical cancer patients with pN0 status is associated with significantly decreased recurrence free survival (1). However, for clinical implementation and for the definition of prognostic threshold levels it is essential to expand this qualitative RT-nested-PCR analysis using a quantitative assay. A disadvantage of qPCR is the high standard deviation observed with low template numbers. We aim to circumvent this problem by using digital PCR (dPCR). This strategy allows the analyses of larger volumes by reducing the unspecific background per template molecule due to reaction partitioning.

Methods

Serial dilutions of 5 ng – 5 pg RNA (corresponding to 500 – 0.5 cells) of the cervical cancer cell line SiHa were prepared in 5 µg RNA of the HPV-negative human keratinocyte cell line HaCaT. Clinical samples consisted of 10 SLN with varying HPV transcript level. Reverse transcription of total RNA (5 µg RNA each) was performed in 100 µl and cDNA aliquots were analysed by qPCR and dPCR. Digital PCR was run in the RainDrop® Digital PCR system (RainDance Technologies) using a probe-based detection of HPV E6/E7 cDNA PCR products with 11 µl template. qPCR was done using a Rotor Gene Q 5plex HRM (Qiagen) amplifying HPV E6/E7 cDNA in a SYBR Green format with 1 µl template.

Results

Both methods showed comparable sensitivity: In the serial SiHa dilutions, qPCR enabled the reliable detection of 0.5 pg template (0.02-0.05 cells) per reaction. Consistently, in dPCR we achieved a detection of 0.55 pg template per reaction. When analysing the sample specific reproducibility, both methods differed considerably. In qPCR, we detected the 50 pg dilution step reliably with variation coefficients between 0.21 and 0.5 throughout the serial dilution series. Using the same samples,

dPCR enabled the detection of the 5 pg dilution step and variation coefficients between 0.04 and 0.5. Generally, we saw with dPCR a substantial reduction of subsampling errors (reduced false negatives). However, in dPCR, the detection of single copies is challenged by the presence of marginal unspecific background signals (1 copy per 275,000 cells).

Conclusion

Compared to real-time PCR, dPCR shows a higher reliability of results while enabling equal sensitivity. The simplicity of the dPCR workflow, with no requirement for a standard curve, and the generation of absolute molecule counts directly from the digital partitions is of particular value for inter and intra-laboratory clinical comparison. These points indicate a high potential of dPCR for the identification and clinical evaluation of occult tumour cells in histologically tumour-free lymph nodes.

References

M. Dürst et al., Prognostic value of HPV-mRNA in sentinel lymph nodes of cervical cancer patients with pN0-status. *Oncotarget* 6, 23015 (Sep 8, 2015)

P16-02

VALIDATION OF A NEW METHOD FOR HIGH RISK HPV DETECTION WITH THE XPERT® HPV ASSAY IN PAP-SPIN™ MEDIUM

C. Cuvelier¹, K. Leybaert¹, E. Van Laere²

¹AZ Alma Hospital, Pathology Department, Eeklo (Belgium), ²AZ Sint Lucas Hospital, Molecular Biology Department, Ghent (Belgium)

Background / Objectives

High-risk human papillomavirus (HR-HPV) infection is one of the most important factors in the development of cervical cancer. The aim of this study is, in combination with cytological screening, to detect the development of high-grade cervical intraepithelial neoplasia in a quick and easy way.

Methods

Liquid-based cervical cytology samples of 32 women in PAP-SPIN™ medium (ThermoScientific – Shandon) were first screened according to the Bethesda classification. Then these samples were tested for HR-HPV with the Xpert HPV® assay (Cepheid), which detects DNA in a cartridge-based RT-PCR-test. The same samples were also tested on the Cobas® HPV Test (Roche) in the laboratory of molecular biology AZ Sint-Lucas Ghent.

GeneXpert is a random-access platform, testing can be completed in 1 hour and the Xpert HPV® assay provides individual HPV16, HPV18/HPV45 genotyping with a simultaneous result for 11 other high-risk HPV genotypes (31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68).

Results

Reproducibility was tested for 10 samples on the Xpert HPV® (1 sample 5x, 8 samples 6x, 1 sample 7x). For these samples, reproducibility is 100%.

Concordance between the Xpert HPV® and Cobas® HPV Test was determined for 31 samples with a result of 93.5% (29/31). Our criterion for the comparison method is > 90%.

In these 31 samples a comparison between cytological screening and Xpert HPV® for HR-HPV was made. Results are as follows:

	Screening	RT-PCR HR HPV
--	-----------	---------------

	Screening	RT-PCR HR HPV				
		Total Positive	Positive for 16	Positive for 18	Positive Others	Negative
RC	15	3			3	12
RC after conisation for min. CIN 2	2	2			2	
ASC-US / AG-C / ASC-H	13	7	2		5	6
LSIL	1					1

Conclusion

HPV detection with the Xpert HPV[®] assay is a simple, reliable and fast method to test the presence of HR-HPV.

P16-03A

Application of Robotic Single-Site Surgery for benign ovarian tumor

S.H. Kwon, H. Chung, E.B. Lee, C.H. Cho, S.D. Cha

Keimyung University, School of Medicine, Daegu, Korea (Korea, republic of)

Background / Objectives

The purpose of this study was to evaluate the feasibility of robotic single-site surgery for benign ovarian tumors with the use of the Da Vinci Si Surgical platform.

Methods

Total five RSS ovarian cystectomy were performed with the Da Vinci Si Surgical platform in a single institution by one surgeon. Data about patient characteristics, indication of surgery, and perioperative outcomes were collected and analyzed.

Results

All procedures were performed successfully. There were three cases of bilateral endometrioma and two cases of unilateral dermoid cyst. Mean operative time was 81.5 minutes (range, 65-95 minutes). No intraoperative or short term postoperative complication of any kind occurred. The median postoperative hospital stay was 2 days.

Conclusion

The use of Da Vinci Si Surgical platform is a feasible and safe treatment approach when used to treat benign ovarian tumors such as dermoid cyst and endometrioma with favorable surgical outcomes. Further evaluation should be performed in a large scale comparative study to confirm benefits of robotic single site surgery in benign ovarian tumor.

P16-03B

Robotic Single-Site Surgery in Carcinoma In Situ of Cervix: A pilot study

S.H. Kwon

Department of Obstetrics and Gynecology, School of Medicine, Keimyung University, Daegu, South Korea (Korea, republic of)

Background / Objectives

Recent reports propose that robotic single-site (RSS) surgery is feasible in treating a benign course of gynecologic processes. The aim of this study is to evaluate the feasibility and safety of RSS surgery for the surgical treatment of early cancerous condition (carcinoma in situ of cervix).

Methods

Patients with preoperative diagnosis of carcinoma in situ (CIS) of cervix by loop electrosurgical excision procedure (LEEP) were selected. 10 patients who underwent robotic single-site surgery from March 2014 to August 2015, at Dongsan medical center, Keimyung University were included in this study. All surgical procedures were performed by robotic single-site instruments (da Vinci Si® surgical System, Intuitive Surgical, Sunnyvale, CA) through a single 2-3 cm umbilical incision. All patients underwent simple hysterectomy with or without salpingo-oophorectomy according to the grossly ovarian pathology.

Results

The Median patient age and body mass index were 42.5 years (range, 33-61 years) and 24.2 kg/m² (range, 18.9-29.0 kg/m²). The median docking time, console time, and total operative time was 10 min (range, 8-15 min), 50 min (range, 36-185 min), and 125 min (range, 90-280 min), respectively. There was no case of conversion to laparoscopy or laparotomy and there was no accessory port insertion. Postoperative wound disruption and dehiscence of umbilical skin occurred in one patient and repaired by non-absorbable suture material under local anesthesia at post-operative 1month.

Conclusion

RSS surgery is feasible and safe in selected patients with early cancerous condition (CIS of Cervix). Operative times were reasonable and surgical procedure was well tolerated by patients. Large-scaled studies comparing laparoendoscopic single site surgery in patients with CIS of cervix should be performed to confirm the safety and benefits of RSS surgery.

P17-01

STUDY PROTOCOL OF THE CHOICE TRIAL: A THREE-ARMED, RANDOMIZED, CONTROLLED TRIAL OF HOME-BASED HPV SELF-SAMPLING FOR NON-PARTICIPANTS IN AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM

M. Tranberg¹, B. Hammer Bech², J. Blaaekær³, J. Skov Jensen⁴, H. Svanholm⁵, B. Andersen⁶

¹Department of Public Health Programs, Randers Regional Hospital, Skovlyvej 1,8930 Randers NØ (Denmark), ²Section for Epidemiology, Department of Public Health, Aarhus University, Bartholins Allé 2, 8000 Aarhus C (Denmark), ³Department of Obstetrics and Gynecology, Aarhus University Hospital, Brendstrupgårdsvej 100, 8200 Aarhus N (Denmark), ⁴Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, (Denmark), ⁵Department of Pathology, Randers Regional Hospital, Skovlyvej 1, 8930 Randers NØ, Department of Public Health Programs, Randers Regional Hospital, Skovlyvej 1,8930 Randers NØ (Denmark), ⁶Department of Public Health Programs, Randers Regional Hospital, Skovlyvej 1,8930 Randers NØ, Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 82, 8200 Aarhus N, Denmark (Denmark)

Background / Objectives

The effectiveness of cervical cancer screening programs is challenged by suboptimal participation and coverage. Offering cervico-vaginal self-sampling for human papillomavirus testing (HPV self-sampling) to non-participants can increase screening participation. However, the effect varies substantially among studies, especially depending on the approach used to offer HPV self-sampling. The present trial evaluates the effect on participation in an organized screening program of a HPV self-sampling kit mailed directly to the home of the woman or mailed to the woman's home on demand only, compared with the standard second reminder for regular screening.

Methods

The CHOICE trial is a parallel, randomized, controlled, open-label trial. It will include 9,327 women aged 30-64 years who are living in the Central Denmark Region and who have not participated in cervical cancer screening after an invitation and one reminder. The women will be equally randomized into three arms: 1) Directly mailed a second reminder including a HPV self-sampling kit; 2) Mailed a second reminder offering a HPV self-sampling kit, to be ordered by e-mail, text message,

phone, or through a webpage; and 3) Mailed a second reminder for a conventional practitioner-collected sample (control group). The primary outcome will be the proportion of women in the intervention groups who participate by returning their HPV self-sampling kit or have a practitioner-collected sample compared with the proportion of women who have a practitioner-collected sample in the control group at 90 days after mail out. Per-protocol and intention-to-treat analyses will be performed. The secondary outcome will be the proportion of women with a positive HPV self-collected sample who attend follow-up testing at 30, 60, or 90 days after mail out of the results.

Conclusion

The CHOICE trial will provide strong and important evidence allowing us to determine if and how HPV self-sampling can be used to increase participation in cervical cancer screening. This trial therefore has the potential to improve prevention and reduce the number of deaths caused by cervical cancer.

P17-02

CONCORDANCE BETWEEN SELF SAMPLING AND PROFESSIONALLY TAKEN CERVICAL HPV TEST-RESULT FROM A POPULATION BASED COHORT STUDY

L. Bergengren¹, **G. Lillsunde Larsson**², **M. Kaliff**², **M.G. Karlsson**², **G. Helenius**²

¹Department of Women's health, Faculty of Medicine and Health, Örebro University (Sweden), ²Department Laboratory Medicine, Faculty of Medicine and Health, Örebro University (Sweden)

Background / Objectives

In Örebro County, Sweden, the organized screening program includes women between 23 and 60. Women with normal cytology exit the program at 55-60 years of age. The aim of this study was to investigate if self-tests for elderly women could be an alternative to professionally taken samples.

Methods

All women (between 55-60) with normal cytology by liquid based cytology (LBC) in their exit sample during the years 2012-2014, a total of 2027, were invited to participate in the study. Retrospectively, all samples, previously biobanked at -25C, were genotyped for HPV with CLART HPV2 (Genomica), which detects 35 different low and high risk genotypes. 247 of 2027 women (12.2%) were positive for any of the 35 HPV genotypes. Of these, 154/247 carried an intermediate or high risk HPV according to the IARC classification, group 1 and 2A and B.

All 154 women were invited for a new cervical sampling, performed by a midwife, after a minimum of 6 months since the exit sample, and where at the same time given a self-sampling device, Rovers Evalyn brush, to use a week after the professional sampling.

Results

A total of 118 of the 154 women returned a self -test. Of these, 66 were positive for a group 1 or 2 genotype. For the professional sampling, the corresponding number was 70 (McNemar test $p=0.557$). 42 of the self-sampled tests were negative for HPV and 10 had low risk genotypes only. Among the professionally collected samples, 40 were negative and 8 positive for low risk types.

The exact same result between professional and self-collected samples was seen in 67/118 samples (56.7%). Of those, 22 harbored an IARC group 1 genotype and 11 an IARC group 2 genotype. 31 samples were unanimously negative for HPV and 9 were concordant multi-infected samples.

Disconcordant results were noted in the remaining 51. Divergence was noted for both high and low risk results between the compared methods. Interestingly, 12 of the group 1 and 2 results in the self-collected samples could not be verified in the professionally collected samples. Also, 13 of group 1 and 2 results in the professional collected samples results were not found in the samples that were self-collected.

Conclusion

Although cervical self-sampling for HPV testing in elderly women should not be solely used, it could be offered as a complement to professionally collected samples.

P17-03

SCREENING OF CERVICAL CANCER IN SENEGAL: A STRATEGY OF INNOVATIVE HEALTH CENTER NABIL CHOUCAIR

O. Gassama, M.E. Faye Diémé, B. Biaye, P.M. Moreira, A. Diouf, J.C. Moreau

Gynecology and Obstetrics, Teaching Hospital Aristide Le dantec, Dakar, Senegal (Senegal)

Background / Objectives

Develop the epidemiological profile of patients who received cervical cancer screening by visual method and colposcopy.

Describe the therapeutic management by resection with snare and histological aspects.

Methods

This was a prospective, descriptive and analytical realized the 06 and June 7, 2015 at the maternity Health Center Nabil Choucair Dakar. All patients had received cervical cancer screening of the uterus by visual methods (IVA / IVL), then a colposcopy in case of positive result. Those in who atypical transformation of Grade 2 or unsatisfactory colposcopy was found had received resection with snare. The surgical specimens were sent in anatomy - pathological.

Results

865 patients were involved in the study. The epidemiological profile of our patient was a multipare of childbearing activity, aged 42 on average, 5 years with an average gestity of 4.4 and an average parity of 3.94. In our series all patients were married and had their first sexual intercourse at 21 years. They used the pill as a contraceptive. 95 patients or 11% had positive results after visual inspection with acetic acid and Lugol applications.

All patients with positive results after application of acetic acid and Lugol had received colposcopy. This colposcopy was normal and satisfactory in 52 cases or 55%, showed a viral colpite in 12 cases or 13%, atypical processing of grade 1 in 8 or 8%, atypical transformation of grade 2 in 21 cases or 22% and 2 cervical polyps or 2%. The 21 patients who had an atypical transformation grade 2 had received the resection snare for diagnostic and therapeutic purposes.

The pathological examination of the cone biopsy revealed piece cervicitis 10 cases or 48%, a condyloma in 3 cases 14%, a CIN2 in 3 cases 14%, and CIN3 in 5 cases or 24%.All were in conizations sano. The postoperative course was uneventful.

Conclusion

Cervical cancer is a real public health problem in developing countries. To human resources, developing countries like Senegal must develop simple strategies; inexpensive, effective globally that must respond to the "screen and treat"

P17-04

HPV-positivity rate in randomised HPV self-sampling study among Slovenian non-responders: comparison of the results between three self-sampling devices

N. Nolde¹, U. Ivanuš², S. Uhan Kastelic¹, T. Jerman², V. Kloboves Prevodnik¹, M. Primic Žakelj²

¹Department of Cytopathology, Institute of Oncology Ljubljana, Slovenia (Slovenia), ²Cervical cancer screening programme and register ZORA, Epidemiology and cancer registries, Institute of Oncology Ljubljana, Slovenia (Slovenia)

Background / Objectives

Non-responders to organised population-based Slovenian cervical cancer screening programme ZORA have higher risk for cervical cancer and also for advanced-stage cancer than regular attendees. With aim to increase the coverage with the screening test among the target population, a randomised controlled HPV self-sampling study was implemented in Slovenia among non-responders aged 30–64 years. The study is still ongoing, it is coordinated by the programme ZORA.

Methods

Women in opt-out arm were randomly allocated to three groups, women in same group received the same self-sampling device (tester) by regular mail: Qvintip® (tester Q, Aprovix AB, Uppsala, Sweden), HerSwab™ (tester H, Eve Medical Inc, Toronto, Canada) or Delphi Screener (tester D, Rovers Medical Devices, Netherlands). Self-taken samples were sent to laboratory by mail where they were assessed for adequacy and analysed with Hybrid capture 2 (HC2). Sample was inadequate if cellularity control in HPV-negative sample was negative. HPV-positivity rate of self-taken samples for women invited to perform self-sampling in the period April – October 2015 was analysed. A multivariate logistic regression model with the type of tester as predictor was run in SPSS 16.0. For some women with HPV-positive self-taken samples also gynaecological-taken sample was obtained in Standard Transport Medium in a colposcopy clinic and analysed with HC2. Positive agreement of self-taken and gynaecological-taken samples was also analysed (positive agreement if woman had both samples positive).

Results

Out of 1866 self-taken samples included in this analysis, 25 (1,3 %) were inadequate. HPV-positivity rate of all adequate self-taken samples was 9,2 % (170/1841), it ranged from 7,5 % (60/804, tester H), to 8,5 % (46/54, tester Q) and 13,0 % (64/493, tester D). The difference in HPV-positivity rate among testers D–H and D–Q was statistically significant ($p < 0,05$). In 64,7 % (110/170) of women with positive self-taken samples also gynaecological-taken sample was obtained and analysed. Average time interval between the two samplings was 52 days. Positive agreement of both samples was 40,9 % (45/110), it ranged from 41,9 % (13/31, tester Q) to 41,7 % (15/36; tester H) and 39,5 % (17/43, tester D). We observed a decrease in positive agreement with increasing time interval between the two samplings, probably due to a regression of HPV infection.

Conclusion

HPV positivity rate of self-taken samples among Slovenian non-responders was 9,2 %. The tester used for self-sampling can be a significant predictor of the HPV-positivity rate. Histopathological correlation will be done to evaluate clinical significance of these results.

P17-05

ANAL CANCER SCREENING: CHARACTERISTICS OF PARTICIPANTS WHO USED TWO HANDS FOR A SELF-ANAL EXAMINATION (SAE)

O. Adegboyega¹, X. Zhang², L.Y. Hwang², S. Baraniuk³, M. White⁴, C. Elizabeth⁵, H. Joseph², O. Nkechi², R. Michael⁶, N. Alan⁷

¹Department of Internal Medicine, Baylor College of Medicine, Houston Texas (United states minor outlying islands), ²Center for Infectious Diseases, Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas (United States of America), ³Coordinating Center for Clinical Trials, Department of Biostatistics, The University of Texas School of Public Health at Houston, Texas (United States of America), ⁴Gordon Crofoot MD PA, Houston, Texas (United States of America), ⁵Department of Internal Medicine, Baylor College of Medicine, Houston, Texas (United States of America), ⁶University of Minnesota, Program in Human Sexuality, Minneapolis, Minnesota (United States of America), ⁷University of Minnesota, Program in Human Sexuality, Minneapolis, Minnesota Center for Infectious Diseases, Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas (United States of America)

Background / Objectives

Anal cancer is primarily caused by human papillomavirus infections and its highest incidence is among men who have sex with men. Since there is no standard screening protocol for anal cancer, a phase II screening study was conducted on the feasibility of self-anal cancer screening. It is important to palpate 360 degrees of the anal canal and we assumed it would require two hands for a single person to accomplish this; thus, participants were encouraged to use two hands for self-palpation of the anal canal if necessary.

Methods

Data for 113 single participants were analysed. Participants who did SAE after training by a clinician were asked, using computer-assisted self-interview, to answer if they palpated the anal canal using two hands or one hand. We then compared the characteristics of those who reported using two hands with those who did not using chi square tests and multivariable analysis.

Results

Slightly less than one-third (31%) of participants used both hands for the SAE. Even so, 83% of men claimed to palpate 360 degrees of the anal canal. Of those who examined themselves with both hands, 73% reported previously inserting fingers into the anus for pleasure compared to 49% for those who used one hand ($p=0.036$). Knowledge of and self-efficacy with SAE technique, education and age were not significantly associated with using two hands. In multivariable analysis, compared to white men, African-American men had 63% lower odds of using both hands for the SAE vs. using one hand.

Conclusion

It is unclear if our assumption of the required use of two hands is correct for all persons, e.g., maybe it is possible to palpate the anal canal with index finger and opposable thumb. Knowing the characteristics of participants who followed directions may be important for tailoring patient education in the next phase of the study. Alternative ways to self-palpate the anal canal should be explored.

P17-06

SELF-SAMPLING IN CIN2+ DETECTION: SENSITIVITY AND SPECIFICITY OF DIFFERENT RLU CUT-OFF IN SPECIMENS FROM 786 WOMEN

F. Bottari¹, **S. Igidbashian**², **S. Boveri**², **A. Tricca**¹, **C. Gulmini**¹, **M. Sesia**¹, **N. Spolti**², **M. Sideri**³, **F. Landoni**², **M.T. Sandri**²

¹Division of Laboratory Medicine, European Institute of Oncology, Milan, Italy (Italy), ²Preventive Gynecology Unit, European Institute of Oncology, Milan, Italy (Italy), ³Deceased (Italy)

Background / Objectives

Mortality for cervical cancer varies between the different regions of the world, with high rates in under developed countries where screening programs are not present and organized. However increasing screening coverage is still a priority in all countries: one way to do that is to base screening on self-sampled screening. The success of a self-sampling screening strategy depends on capacity to recruit unscreened women, on the performance and acceptability of the device, and on the clinical performance of the high-risk HPV test.

Methods

This study based on 786 women scheduled for cervical cytology for any reason or for a cervical conservative treatment for CIN at European Institute of Oncology, investigates the best cut-off value of HC2 (Qiagen, Hilden, Germany) test, a signal amplification detection system based on chemiluminescence that detects 13 HR-HPV, for self-sampled specimens (Qiagen Hybrid Capture (HC) Cervical Sampler) in terms of sensitivity and specificity.

Results

In our population we found that the sensitivity and the specificity for cervical intraepithelial neoplasia grade 2 or more (CIN2+) detection of HC2 performed on self-sampled specimens were 82.5% and 82.8% respectively, considering the relative light units (RLU) cut-off of 1. Increasing the cut-off value the sensitivity decreases and the specificity raises and the best AUC for the RLU cut-off is 1.

Conclusion

Our results confirms that the 1 cut-off suggested by Qiagen for PreservCyt specimen is the best cut-off also for self-sampled specimens.

P17-07

PRELIMINARY EVALUATION OF CERVICAL CANCER SCREENING BY CYTOLOGY AND HPV TESTING IN NORTH-WESTERN REGION OF ROMANIA

O. Suteu¹, F. Nicula¹, R. Pai?¹, M. Bumbu¹, D. Coza¹, L. Blaga¹, B. Nicula¹, A. Itu¹, A. Todescu¹, E. Weiderpass²

¹„Prof. Dr. Ion Chiricuta” Institute of Oncology, Cluj-Napoca (Romania),

²Research Department, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland; Department of Commun (Norway)

Background / Objectives

Background. A national cervical cancer screening program was started in Romania in September 2012, financed by the Ministry of Health. The program is based on conventional cytology. HPV testing is not offered in the public health sector. A pilot study was conducted in the North-Western Region of Romania, aiming to evaluate the feasibility of integrating HPV high risk (hr-HPV) as primary test into the screening program. We present here preliminary results of co-testing with cervical cytology and hr-HPV.

Objectives: A) To estimate the prevalence of HPV infection and abnormal cytology in an ethnically diverse screening population; B) To assess the consistency of screening results using hr-HPV and cytology.

Methods

Methods. A cross-sectional pilot study was conducted. The target population was Roma, other ethnic minorities and other socioeconomic disadvantaged groups from the rural population in the North-Western Region of Romania. Cytological smears and hr-HPV tests were taken concurrently in each woman between June and November 2015 by a mobile health unit. The specimens were tested with Hybrid Capture 2 DNA Test for the qualitative detection of 13 hr-HPV types (1rlu/co cut-off). Positive cytology was considered ASC or worse. Screen positivity rates and agreement between cytology and hr-HPV were estimated by kappa coefficient.

Results

Results. 1049 women were included in the study. Their mean age was 44 years (range 20-64 years). Most participants were Romanian (74%), the remaining being Roma (19%), Ukrainian (6%) and Slovakian (2%). The prevalence of hr-HPV was 14% among the Roma, 12% among Ukrainian, 12% among Romanian and 10% among Slovakian women. The prevalence of positive cytology was 14% among Romanian, 7% among Ukrainian, and 5% among Roma women. The population prevalence of positive results was similar for both hr-HPV test and cytology (12%). However, 66% of the hr-HPV positive women had normal cytology and 8% of women had an abnormal cytology with hr-HPV negative test. The consistency of positive results using the two methods was fair ($\kappa=0.25$; 95% CI=0.18 to 0.30, $p<0.001$). A total of 208 women (20%) were positive by one or two tests, while only 41 (4%) were positive for both tests. 100% of HSIL cases were HPV positive, 50% for LSIL, 34.2% for ASC-H, 26% for ASCUS and 20% for AGC.

Conclusion

Conclusions. This is the first study in Romania to assess the prevalence of HPV infection and abnormal cytology in an ethnically diverse and socioeconomic disadvantaged screening population. Our data indicate that co-testing is feasible for earlier identification of high risk groups for cervical cancer in this context.

P17-08

IS THERE EVIDENCE FOR CERVICAL CANCER SCREENING IN ELDERLY WOMEN?

L.H. Thamsborg¹, M. Waldstrøm², H. Svanholm³, C. Rygaard⁴, E. Lynge¹

¹Department of Public Health; University of Copenhagen (Denmark), ²Pathology Department, Vejle Hospital (Denmark), ³Pathology Department, Randers Hospital (Denmark), ⁴Pathology Department, Hvidovre Hospital (Denmark)

Background / Objectives

In Denmark, screening for cervical cancer with liquid-based cytology (LBC) is offered every three years in the age-group 23-49 years, and every five years in women aged 50-64 years. For women aged 60-64 years cytology screening can be replaced by an HPV- DNA test, and the woman is checked-out of the screening programme if she is HPV negative. At present, the age-specific incidence of cervical cancer in Denmark shows a bipolar pattern with peaks both in younger and older ages. A considerable part of new cases and deaths from cervical cancer occur in women over 60 years of age. It has, therefore, been suggested to increase the upper age-limit for cervical cancer screening.

Methods

We analyzed incidence and mortality from cervical cancer and data on screening participation in women 64+ years. Data on incidence and mortality were extracted from Danish and Nordic registers. Data on screening participation were obtained from the Central Denmark Region, where 8,868 women 64+ years were invited to screening in 2014 to remedy a previous mistake in the invitation software.

Results

The number of incident cervical cancer cases decreased from an average of 860 per year in 1958-62 to 372 in 2008-12, and the number of cervical cancer deaths decreased from an average of 320 per year in 1958-62 to 98 in 2008-12. During the same period, the age-standardized incidence (Nordic Standard Population) for women 60+ years decreased from 45.7 to 16.8 per 100,000 and mortality from 28.8 to 8 per 100,000. The relative share of cervical cancer cases in women 60+ years increased from 21% in 1958-62 to 30% in 2008-12. Analysis of incidence by birth cohort indicated that younger generations are expected to experience a considerably lower incidence level in older ages than the

level seen at present in elderly women. Out of the 8,868 women 64+ years invited to screening in 2014, 11.3% participated and 5.5% of the participants were high-risk HPV-positive.

Conclusion

Today one third of new cervical cancer cases and two thirds of cervical cancer deaths occur in women 60+ years. However, the pattern we see today is a result of different cervical cancer risks across birth cohorts. The current incidence peak of cervical cancer in elderly women could motivate a one-time offer of HPV screening of women aged 65-79, but the evidence does not support a permanent change in the present screening programme because future generations of elderly women will have a lower risk of cervical cancer.

P17-09

CURRENT STANDARDS AND OPTIONS FOR NATIONAL CERVICAL CANCER SCREENING PROGRAMME IN CROATIA

J. Zekan¹, D. Karelovic², H. Haller³, D. Nakic⁴, J. Juras¹, J. Lesin¹

¹Department of Gynaecologic Oncology University Hospital Centre Zagreb, Petrova 13, 10000 Zagreb, Croatia (Croatia), ²Department of Gynaecology and Obstetrics University Hospital Centre Split, Spinciceva 1, 21000 Split, Croatia (Croatia), ³Department of Gynaecology and Obstetrics University Hospital Centre Rijeka Kresimirova 42, 51000 Rijeka, Croatia (Croatia), ⁴Croatian Institute of Public Health, Rockefellerova 7, 10000 Zagreb, Croatia (Croatia)

Background / Objectives

Since the middle of the last century, opportunistic screening was implemented in the Republic of Croatia, and this led to a reduction in the incidence and mortality of cervical cancer. However, with the opportunistic programme it is not possible to reduce the incidence of disease under 10/100.000, since most of the new cases arise from the part of the population that is not covered with screening. Ministry of Health of the Croatian Government started in December 2012 implementation of national organised cervical cancer screening programme. The objectives of the programme are to decrease the incidence of invasive cervical cancer by 60% in the age group 25-65 years after 8 years from the beginning of the programme, reducing mortality by 80% in the age group 25 to 70 years after 13 years and gradual cessation of opportunistic screening.

Methods

The organisation of the national programme includes the creation of a database of the target population, organization of the activate invitations (call/recall system) and establishment of follow-up protocol and monitoring. The target population is all asymptomatic women aged 25 to 65. Screening interval is 3 years, which means the invitation of about 400,000 women annually. Screening test is conventional Pap test, which is conducted according to the guidelines given by gynaecological and cytological professional associations. The predictive value of the screening method can be raised by introducing HPV molecular detection test. HPV tests, which detect HPV infections associated with cervical cancer, can forecast cervical cancer risk many years in the future and are currently recommended to be used in conjunction with the Pap test in some women, either as an additional screening test or when Pap test results are uncertain.

Conclusion

Most cervical precancers develop slowly, so cancer can usually be prevented if a woman is screened regularly. We expect to improve the quality of the programme by introducing HPV testing. Invasive disease ranging from 50% (meta-analysis) to 70% at best. The predictive value of the screening method can be raised by introducing HPV molecular detection test. HPV tests, which detect HPV infections associated with cervical cancer, can forecast cervical cancer risk many years in the future and are currently recommended to be used in conjunction with the Pap test in some women, either as an additional screening test or when Pap test results are uncertain. HPV tests can also identify women at risk for an uncommon type of cervical cancer (adenocarcinoma) that is often missed by Pap tests.

P17-10

Clinical value of fully automated Ki67/p16 dual staining as biomarkers for triage of HPV positive women in a screening program

I.T.Ø Øvestad

Stavanger University Hospital (Norway)

Background / Objectives

The use of Ki67/p16 dual staining of cytology smears as a prognostic marker for identifying high grade Cervical Intraepithelial Neoplasia (CIN) has been suggested. In a group of women attending the Norwegian Cervical Cancer Screening Program, an automated staining protocol was used to test the clinical value of Ki67/ p16 dual staining alone or as an adjunct to cytology.

Methods

The CINtec PLUS kit was used for dual staining of liquid based PAP smears in residual specimens after cytology testing. The results were correlated with CIN grade 1, 2 or 3 in biopsies or HPV negative cytology as the clinical endpoint on average 184 days after cytology specimen collection.

Results

In a total of 266 HPV positive women, 67% were CINtec PLUS positive. For detecting CIN2+ in the whole cohort (201) or the subgroup of non HPV16 or 18 positives (136), the sensitivity for CINtec PLUS was significantly higher than for cytology (0.88 versus 0.79), but not for CIN3 (0.94 versus 0.88). The specificity of cytology to detect \leq CIN2 was significantly higher than for CINtec PLUS (0.35 versus 0.28), but not for detecting \leq CIN1 (0.35 versus 0.31). Using the CINtec PLUS as an adjunct to cytology increased the sensitivity for CIN3 (0.96) and CIN2+ (0.94), while the specificity was higher for CIN3 (0.40) and lower for CIN2+ (0.24).

Conclusion

Dual staining of p16 and ki67 as an adjunct to cytology is more sensitive and more specific to use for detection of CIN3, but less specific for CIN2+, as compared to CINtec PLUS or cytology alone.

P17-11

THE EFFECT OF ANAL CANCER AWARENESS AND ANXIETY ON SCREENING IN MEN WHO HAVE SEX WITH MEN

J. Hicks¹, **L.Y. Hwang**², **M.S. Baraniuk**³, **M. White**⁴, **E. Chiao**⁵, **N. Onwuka**¹, **M. Ross**⁶, **A. Nyitray**⁶

¹Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas, USA. (United States of America), ²Center for Infectious Diseases, Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health at Houston, Texas, USA. (United States of America), ³Coordinating Center for Clinical Trials, Department of Biostatistics, The University of Texas School of Public Health at Houston, Texas, USA (United States of America), ⁴Gordon Crofoot MD PA, Houston, Texas, USA (United States of America), ⁵Department of Internal Medicine, Baylor College of Medicine, Houston, Texas, USA. (United States of America), ⁶University of Minnesota, Program in Human Sexuality, Minneapolis, Minnesota, USA. (United States of America)

Background / Objectives

Men who have sex with men (MSM) are at greater risk of developing anal cancer caused by human papillomavirus (HPV) than the rest of the general population. Currently, no formal national guidelines exist advising men about anal cancer screening in the United States. Studies show that MSM's knowledge of anal cancer and HPV is quite low. We sought to assess differences in demographics, familiarity and anxiety about anal cancer between those men who reported having had anal cancer screening and those who did not.

Methods

155 MSM in Houston, Texas were recruited to participate in a study to assess the feasibility of teaching self- and partner-assisted anal examinations as a means of screening for anal cancer. Data were obtained during eligibility screening, a written pre-test and a computer-assisted self-interview.

Results

Of the 155 participants, 106 (68.4%) reported having had anal cancer screening in their life. Men who reported anal cancer screening tended to be positive for HIV ($p = 0.04$). Men who did not report anal

cancer screening were more likely to be African American ($p = 0.04$) and reported knowing less about anal cancer ($p = 0.01$). Of 129 men who were directly asked if they had had anal cancer screening, 61 (47.3%) men said they had not, despite reporting a history of an anal Pap test or a digital rectal exam.

Conclusion

Though MSM who have been screened are more confident in their knowledge of anal cancer, many are not aware of what comprises anal cancer screening.

P17-12

FOCALPOINT COMPUTER-ASSISTED PAP TEST SCREENING: VALIDATION TECHNOLOGY STUDY FOR IMPLEMENTATION IN A PUBLIC HEALTH SERVICE

A. Longatto-Filho¹, J.E. Levi², T.R. Martins³, D. Dede Cohen⁴, L.M. Fuza⁴, L. Cury⁴, L. Lina-Villa³, J. Eluf-Neto³

¹Laboratory of Medical Investigation (LIM14), Department of Pathology of Faculdade de Medicina da Universidade de São Paulo (USP's College of Medicine), São Paulo, Brazil (Brazil), ²Instituto de Medicina Tropical da Universidade de São Paulo (USP's Institute of Tropical Medicine), Virology Laboratory; São Paulo, Brazil (Brazil), ³Faculdade de Medicina da Universidade de São Paulo (USP's College of Medicine), São Paulo, Brazil (Brazil), ⁴Fundação Oncocentro de São Paulo, São Paulo, Brazil (Brazil)

Background / Objectives

Pap test slides evaluated by computer-assisted methodology is believed to be more precise than manual reading. We validate the use of computer-assisted technology for Pap test reading for implementation in a public health service.

Methods

We evaluated prospectively 12,084 PapTest from women examined at Fundação Oncocentro de São Paulo, with median age of 42 years old. The cervical samples were preserved in BD-SurePath liquid medium and prepared in BD Totallys equipment. The slides were primarily read by Focalpoint system (BD, Burlington, USA) and afterwards 10 selected fields were revised by well-trained cytotechnologists and cytopathologists.

Results

Manual slide reading identified 112 (0.9%) ASC-H, 329 (2.7%) LSIL, 70 (0.6%) HSIL and 2 (0.00%) invasive squamous cells carcinoma. Focalpoint quintiles classified the two invasive cancer in Q1 and almost 90% of HSIL and almost 85% of ASC-H in in Q1 and Q2, respectively. One H1SL was classified as Q5 and detected in manual reading. Negative Pap test results were similarly distributed among all quintiles. Miscellaneous accounted 890 (7.4%).

Conclusion

Computer-assisted screening can drastically reduce the need for manual Pap test reading, which may safely increase productivity.

References

1. Rebolj M, Rask J, van Ballegooijen M, Kirschner B, Rozemeijer K, Bonde J, Rygaard C, Lynge E. Cervical histology after routine ThinPrep or SurePath liquid-based cytology and computer-assisted reading in Denmark. *Br J Cancer*. 2015 Nov 3;113(9):1259-74.
2. Bowditch RC, Clarke JM, Baird PJ, Greenberg ML. Morphologic analysis of false negative SurePath® slides using Focalpoint™ GS computer-assisted cervical screening technology: An Australian experience. *Diagn Cytopathol*. 2015 Nov;43(11):870-8.
3. Stein MD, Fregnani JH, Scapulatempo-Neto C, Longatto-Filho A. Cervicovaginal cytology in patients undergoing pelvic radiotherapy using the Focalpoint system: results from the RODEO study. *Diagn Pathol*. 2015 Jan 16;10:1.
4. Stein MD, Fregnani JH, Scapulatempo C, Mafra A, Campacci N, Longatto-Filho A; RODEO Study Team From Barretos Cancer Hospital. Performance and reproducibility of gynecologic cytology interpretation using the FocalPoint system: results of the RODEO Study Team. *Am J Clin Pathol*. 2013 Oct;140(4):567-71.

P18-01

The vaginal microbiota of women with threatened pregnancy who subsequently have ongoing pregnancy versus those who subsequently have miscarriage in the first trimester of gestation

A. Akhmedova, G. Totchiyev

People's Friendship University of Russia (Russian federation)

Background / Objectives

Vaginal infections are one of the main reasons of miscarriage. This study was undertaken to determine the vaginal microbiota of patients with threatened pregnancy who subsequently had ongoing pregnancy versus those who subsequently had miscarriage at the first trimester of gestation.

Methods

This was a study of 87 pregnant women attending hospital because of a threatened pregnancy at the first trimester of gestation. The women were divided into 2 groups: 1) 33 women with threatened pregnancy who subsequently had miscarriage; 2) 54 women with threatened pregnancy who were discharged from the hospital with ongoing pregnancy. Samples of vaginal swabs were collected from all investigated women before any treatment to perform quantitative polymerase chain reaction (qPCR) for microbial species assessment and vaginal pH evaluation. Fluorescence in situ hybridization (FISH) was also used to investigate microbiota of desquamated epithelial cells of vagina from urine sediment.

Results

Vaginal dysbiosis was detected in 12/33 (36,4%) women of the 1st group and 24/54 women (44,4%) of the 2nd group ($pH > 4,5$). All these 36 women had moderate vaginal dysbiosis (*Lactobacillus* spp. 20-80%). Statistical differences between 2 groups ($p < 0,05$) were detected in the quantity of *Lactobacillus* spp. ($28 \pm 6,2\%$ and $75 \pm 9,6\%$ respectively), *Gardnerella vaginalis* ($60 \pm 5,0$ and $45 \pm 7,3\%$ respectively), *Atopobium vaginae* ($60 \pm 6,5\%$ and $45 \pm 6,9\%$ respectively), and Enterobacteriaceae ($15 \pm 4,9\%$ and $2 \pm 0,5\%$ respectively). FISH enabled us to see *Gardnerella* biofilms but no statistical differences were found between 2 groups of patients ($p > 0,05$). That was 3/33 (9,1%) in the 1st group and 12/54 (22,2%) in the 2nd group.

Conclusion

Threatened pregnancy was characterized with moderate vaginal dysbiosis. The number of *Lactobacillus* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, and *Enterobacteriaceae* was statistically higher in women with threatened pregnancy who subsequently had miscarriage versus those who had ongoing pregnancy in the first trimester of gestation. No statistical difference was found in the number of *Gardnerella* biofilms between women with threatened pregnancy either with subsequent ongoing pregnancy or with miscarriage.

References

1. Lamont RF, Sobel JD, Akins RA, et al. The vaginal microbiome: New information about genital tract flora using molecular based techniques. *BJOG: an international journal of obstetrics and gynaecology*. 2011;118(5):533-549. doi:10.1111/j.1471-0528.2010.02840.x.
2. Andosova LD, Kontorshchikova KN, Kachalina OV, Belov AV, Gonova ES, Kudel'kina Slu. The characteristic of biocenosis of urogenital tract in women. *Klin Lab Diagn*. 2013 Jan;(1):51-3.
3. Sangkomkarn US, Lumbiganon P, Prasertcharoensuk W, Laopaiboon M. Antenatal lower genital tract infection screening and treatment programs for preventing preterm delivery. *Cochrane Database of Systematic Reviews* 2015, Issue 2. Art. No.: CD006178. DOI: 10.1002/14651858.CD006178.pub3.
4. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 2011, 108(Suppl 1):4680–4687.
5. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, Gevers D, Huttenhower C, Petrosino J, Versalovic J: A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One* 2012, 7:e36466.
6. Cartwright CP, Lembke BD, Ramachandran K, Body BA, Nye MB, Rivers CA, Schwebke JR. 2012. Development and validation of a semi-quantitative, multitarget PCR assay for diagnosis of bacterial vaginosis. *J. Clin. Microbiol.* 50:2321–2329.
7. Datcu R, Gesink D, Mulvad G, Montgomery-Andersen R, Rink E, Koch A, Ahrens P, Jensen JS. 2013. Vaginal microbiome in women from Greenland assessed by microscopy and quantitative PCR. *BMC Infect. Dis.* 13:480.
8. Shipitsyna E, Roos A, Datcu R, Hallen A, Fredlund H, Jensen JS, Engstrand L, Unemo M. 2013. Composition of the vaginal microbiota in women of reproductive age—sensitive and specific molecular diagnosis of bacterial vaginosis is possible? *PLoS One* 8:e60670.
9. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosch DW, Nikita L, Galuppi M, Lamont RF, Chaemsaihong P, Miranda J, Chaiworapongsa T, Ravel J: The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014, 2:4

10. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Bieda J, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with abnormal delivery at term. *Microbiome* (2014) 2:18. doi:10.1186/2049-2618-2-18
11. Datcu R, Gesink D, Mulvad G, Montgomery-Andersen R, Rink E, Koch A, Ahrens P, Jensen JS. Bacterial vaginosis diagnosed by analysis of first-void-urine specimens. *J Clin Microbiol*. 2014 Jan;52(1):218-25. doi: 10.1128/JCM.02347-13. Epub 2013 Nov 6. PMID: 24197876
12. Swidsinski A, Dorffel Y, Loening-Baucke V, Schilling J, Mendling W: Response of *Gardnerella vaginalis* biofilm to 5 days of moxifloxacin treatment. *FEMS Immunol Med Microbiol* 2011, 61:41–46.
13. Machado A, Almeida C, Salgueiro D, Henriques A, Vanechoutte M, Haesebrouck F, Vieira MJ, Rodrigues L, Azevedo NF, Cerca N. Fluorescence in situ hybridization method using peptide nucleic acid probes for rapid detection of *Lactobacillus* and *Gardnerella* spp. *BMC Microbiology*. 2013;13:82. doi: 10.1186/1471-2180-13-82.
14. Machado A, Castro J, Cereija T, Almeida C, Cerca N. Diagnosis of bacterial vaginosis by a new multiplex peptide nucleic acid fluorescence in situ hybridization method. Smidt H, ed. *PeerJ*. 2015;3:e780. doi:10.7717/peerj.780.
15. Swidsinski A, Loening-Baucke V, Mendling W, Dörffel Y, Schilling J, Halwani Z, Jiang XF, Verstraelen H, Swidsinski S. Infection through structured polymicrobial *Gardnerella* biofilms (StPM-GB). *Histol Histopathol*. 2014 May;29(5):567-87. Epub 2013 Dec 11.
16. Hardy, L., Jaspers, V., Dahchour, N., Mwambarangwe, L., Musengamana, V., Vanechoutte, M., & Crucitti, T. (2015). Unravelling the Bacterial Vaginosis-Associated Biofilm: A Multiplex *Gardnerella vaginalis* and *Atopobium vaginae* Fluorescence In Situ Hybridization Assay Using Peptide Nucleic Acid Probes. *PLoS ONE*, 10(8), e0136658. <http://doi.org/10.1371/journal.pone.0136658>
17. Van Acker H, Van Dijck P, Coenye T (2014) Molecular mechanisms of antimicrobial tolerance and resistance in bacterial and fungal biofilms. *Trends Microbiol* 22(6):326–333
18. Verstraelen H., Swidsinski A. The biofilm in bacterial vaginosis: Implications for epidemiology, diagnosis and treatment. *Curr. Opin. Infect. Dis* Year: 2013; 26: 86-89
19. Hemalatha R, Ramalaxmi BA, Swetha E, Balakrishna N, Mastromarino P. Evaluation of vaginal pH for detection of bacterial vaginosis. *The Indian Journal of Medical Research*. 2013;138(3):354-359.
20. Krauss-Silva L, Almada-Horta A, Alves MB, Camacho KG, Moreira MEL, Braga A. Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. *BMC Pregnancy and Childbirth*. 2014;14:107. doi:10.1186/1471-2393-14-107.
21. Foroozanfard F, Tabasi Z, Mesdaghinia E, Sehat M, Mehrdad M. Cervical length versus vaginal PH in the second trimester as preterm birth predictor. *Pakistan Journal of Medical Sciences*. 2015;31(2):374-378. doi:10.12669/pjms.312.6310.

P18-02

The prevalence of human papillomavirus infection and other sexually transmitted infections in the anus of Japanese men who have sex with men: A molecular and cytological analysis

H. Iwamoto¹, **K. Shigehara**¹, **H. Yaegashi**², **K. Nakashima**¹, **S. Kawaguchi**¹, **I. Itoda**³, **T. Sasagawa**⁴, **M. Namiki**¹

¹Department of Urology, Kanazawa University Graduate School of Medical Science (Japan), ²Department of Urology, Ishikawa Prefectural Central Hospital (Japan), ³Shirakaba Clinic (Japan), ⁴Department of Reproductive and Perinatal Medicine, Kanazawa Medical University (Japan)

Background / Objectives

Recently, potential associations between anal carcinoma and human papillomavirus have been proposed. We investigated prevalence of human papillomavirus (HPV), Chlamydia trachomatis (CT), Neisseria gonorrhoea (NG), Mycoplasma genitalium (MG), Mycoplasma hominis (MH), and Ureaplasma (U) spp. in the anal samples among Japanese men who have sex with men (MSM). In addition, we evaluate cytological findings to find the association with HPV infection.

Methods

150 MSM aged 24-59 years-old (mean: 40.7 years old) were enrolled in the present study. Anal rubbed samples were collected, and HPV, CT, NG, MG, MH, and U spp. were examined based on the polymerase chain reaction assay. β -globin amplification was also checked to confirm the quality of DNA sample. The HPV genotype was determined by using HPV GenoArray kit (Hybri-Max™). In addition, cytological evaluation was performed in each sample. We investigated the associations between HPV infection and cytological findings.

Results

Human immunodeficiency virus (HIV) infection rate among participants was 96%. All HIV positive participants had been receiving anti-retroviral therapy. Beta-globin was positive in 88.7% (133/150), and HPV detection rate were 79.7% (106/133). HPV16 was the most common type in anal samples. The most common was CT, found in 14.3%, followed by MH (6%), U (5.3%), NG (4.5%) and MG (3.8%) in anal samples. HPV, high-risk HPV and other STIs detection rate rises significantly as the anal cytology dysplasia grade increases. Multivariate regression analysis found that the latest receptive

anal intercourse within 6 months was an independent risk factor for high-risk HPV infection and other STIs.

Conclusion

HPV and several microorganisms were detected from anus of MSM. In particular, anal HPV and high-risk HPV and other STIs detection rate were significantly associated with cytological abnormality. HPV infection-associated cytological abnormality suggests that persistent HPV infection for MSM may result in the development of anal carcinoma, similar to cervical cancer caused by HPV infection in women.

References

- 1) Mork J, et al. Human papillomavirus infection as a risk for squamous-cell carcinoma of the head and neck. *N Engl.J.Med.* 2001;344:1125-31
- 2) Shigehara K, et al. Etiologic role of human papillomavirus infection in bladder carcinoma. *Cancer.* 2011;117:2067-76
- 3) Zur, Hausen, H. Papillomavirus infections: a major cause of human cancers. *Biochem. Biophys. Acta.*1996 ; 1288 : F55-78.
- 4) Smith JS, et al. Age-specific prevalence of infection with human papillomavirus in females: a global review. *J. Adolesc Health* 2008 ; 43 : S5–S25.
- 5) Dunne, EF, et al. Prevalence of HPV infection among men: A systematic review of the literature. *J Infect Dis.* 2006 ; 194 (8) : 1044-57.
- 6) Schim van der Loeff MF, et al. HPV and anal cancer in HIV-infected individuals: a review. *Curr HIV/AIDS Rep.* 2014;11:250-62.
- 7) Zaki SR, et al. Human papillomavirus infection and anal carcinoma. Retrospective analysis by in situ hybridization and the polymerase chain reaction. *Am. J. Pathol.* 1992;140:1345-55.
- 8) Kiviat NB, et al. Association of anal dysplasia and human papillomavirus with immunosuppression and HIV infection among homosexual men. *AIDS.* 1993;7:43-9.
- 9) Palefsky JM, et al. Risk factors for anal human papillomavirus infection and anal cytologic abnormalities in HIV-positive and HIV-negative homosexual men. *J Acquir. Immune. Defic. Syndr.* 1994;7:599-606.
- 8) Grulich AE, et al. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet.* 2007;370:59-67.

- 10) Hernandez AL, et al. Risk factors for anal human papillomavirus infection type 16 among HIV-positive men who have sex with men in San Francisco. *J.Acquir.Immune.Defic.Syindr.* 2013;63:532-9.
- 11) Jong E, et al. Effect of HIV viral load, CD4 cell count and antiretroviral therapy on human papillomavirus prevalence in urine samples of HIV-infected men. *Int.J.STD AIDS.* 2009;20:262-4.
- 12) Costa FA, et al. Prevalence of human papillomaviruses in urine samples of male patients infected with HIV-1 in Sao Pulo, Brazil. *J.Med.Virol.* 2009;81:2007-11.
- 13) Kawaguchi S, et al. Liquid-based urine cytology as a tool for detection of human papillomavirus, Mycoplasma, and Ureaplasma in men. *J. Clin. Microbiol.* 2012;50:401-406.
- 14) Shigehara, et al. Prevalence of human papillomavirus infection in the urinary tract of men with urethritis. *Int. J. Urol.* 2010;17:563-568.
- 15) Palefsky JM, et al. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J.Infect.Dis.* 1998;177:361-7.
- 16) Parisi SG, et al. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. *BMC Infect.Dis.* 2011;11:150.
- 17) Phanuphak N, et al. Anal human papillomavirus infection among Thai men who have sex with men with and without HIV infection: prevalence, incidence, and persistence. *J.Acquir.Immune.Defic.Syindr.* 2013;63:472-9.
- 18) Fuchs W, et al. Asymptomatic anal sexually transmitted infections in HIV-positive men attending anal cancer screening. *Br J Dermatol.* 2015 Nov 17. doi: 10.1111/bjd.14288.

P19-01

Giant condyloma accuminatum mimicking vulvar verrucous carcinoma

F. Ghaemmaghaminori

Tehran University of Medical Science (Iran, islamic republic of)

Background / Objectives

A 46-year-old woman Gravida: 1 Para: 1 presented with a huge mass on her external genitalia unresponsive to local medical treatments including podophyllin solution and trichloroacetic acid. Her physician had recommended surgical resection but she refused initially.

Methods

She had pemphigus vulgaris and diabetes mellitus and was under treatment with prednisolone and glybenclamide.

Examination revealed a 20 × 15 cm cauliflower-like tumor mass involving the mons pubic, the labia majores, the perineum and medial aspect of left thigh (Fig. 1).

Display Full Size version of this image (43K)

.

Treponema pallidum hemagglutination assay and test for antibody to human immunodeficiency virus and hepatitis B virus antigen were negative.

She was treated with intravenous cefazolin and metronidazole for infection.

Multiple biopsies detected ordinary condyloma accuminatum, and neither malignancy nor cytological atypia was detected. HPV DNA analysis by polymerase chain reaction revealed HPV type 6.

Wide surgical excision was performed. Histopathologic examination showed multiple papillary structures covered by stratified keratinized squamous epithelium and koilocytic changes with fibrovascular core and infiltration of predominantly mononuclear inflammatory cells into the vascular core. No significant squamous atypia was seen and verrucous carcinoma was excluded. The histopathology was typical for a condyloma. The postoperative course was uneventful and the cosmetic result was satisfactory

Results

Giant condyloma has a benign histologic appearance. It resembles that of condyloma acuminatum, and it may be difficult to distinguish them from each other.¹

A large representative biopsy specimen is important to judge the structure of the lesion in order to establish the diagnosis and to exclude foci of verrucous carcinoma. Giant condyloma invades by expansion rather than by infiltration, leaving basement membrane intact, and shows a well-stratified epithelium with minimal cellular dysplasia or atypical cells.²

Conclusion

Giant condyloma has been treated by a variety of modalities. However, literature consists mainly of case reports and lacks controlled studies.³ and 4 A study on 42 published cases concluded that the only consistently effective therapy is wide surgical excision of tumor with clear margins with or without adjuvant chemotherapy.⁵

Radiation therapy is controversial and should only be considered in patients with non-resectable tumors as anaplastic transformation may be induced

Patients with extensive lesions may require systemic chemotherapy with a variety of combinations of methotrexate, 5-fluorouracil, bleomycin, mitomycin C, cisplatin, and leucovorin.⁹

References

References

- 1 H. Yag, M. Igawa and H. Shiina et al., A study of growth pattern in giant condyloma acuminatum, *Urol Int* 61 (1998), pp. 188–191.
- 2 A. Geusau, G. Heinz-peer and B. Volc-platzer, Regression of deeply infiltrating giant condyloma (Buschke–Lowenstein tumor) following long term intralesional interferon alfa therapy, *Arch Dermatol* 136 (2000), pp. 707–710. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (15)
- 3 L. Zhao, F. Fang and F. Carey et al., A case of condyloma acuminatum with giant and multiple lesions on the vulva and breast: successfully treated with surgical operations, *Int J STD AIDS* 15 (Mar 2004) (3), pp. 199–201.

- 4 F.C. Antony, M. Ardern-Jones and A.V. Evans et al., Giant condyloma of Buschke–Loewenstein in association with erythroderma, *Clin Exp Dermatol* 28 (2003), pp. 46–49. [Full Text via CrossRef](#) | [View Record in Scopus](#) | [Cited By in Scopus \(6\)](#)
- 5 Q.D. Chu, M.P. Vezeridis and P.N. Libbey et al., Giant condyloma acuminatum (Buschke–Loewenstein tumor) of the anorectal and perianal regions: analysis of 42 cases, *Dis colon Rectum* 37 (1994), pp. 950–957. [Full Text via CrossRef](#) | [View Record in Scopus](#) | [Cited By in Scopus \(40\)](#)
- 6 C.W. Sobrado, M. Mester and W. Nadalin et al., Radiation-induced total regression of a highly recurrent giant perianal condyloma, *Dis colon Rectum* 43 (2000), pp. 257–260. [Full Text via CrossRef](#) | [View Record in Scopus](#) | [Cited By in Scopus \(13\)](#)
- 7 K. Uchida, H. Safani and T. Okuno et al., Two cases of giant condyloma of the penis treated with penis-sparing methods, *Hinyokika Kyo* 42 (1996), pp. 605–608. [View Record in Scopus](#) | [Cited By in Scopus \(2\)](#)
- 8 D.G. Hatzichristou, A. Apostolidis and V. Tzotzis et al., Glanslectomy: an alternative surgical treatment for Buschke–Lowenstein tumor of penis, *Urology* 57 (2001), pp. 966–969. [SummaryPlus | Full Text + Links](#) | [PDF \(246 K\)](#) | [View Record in Scopus](#) | [Cited By in Scopus \(16\)](#)
- 9 G. Persson, K. Andersson and I. Krantz, Symptomatic genital papillomavirus infection in a community B incidence and clinical picture, *Acta Obstet Gynecol Scand* 75 (1996), pp. 287–290. [View Record in Scopus](#) | [Cited By in Scopus \(21\)](#)

P19-02

ADVANCED VULVAR CANCER. TWO DIFFERENT CASES OF THE SAME DISEASE

A.M. Serrano, I. Chelea, E. Cazorla

HOSPITAL UNIVERSATARIO DE TORREVIEJA (Spain)

Background / Objectives

Description of two cases of advanced vulvar cancer in patients with different profiles and pathology results, and outline of the treatment strategies for each of them.

Methods

The first case describes a 62 year-old woman admitted with ketoacidosis who had not attended a doctor in years. She was admitted to the intensive care unit, and Gynaecology staff were asked to assess a vulvar lesion. On assessment, a lesion was found covering the right labia minora and majora, with presence of tissue necrosis and stench, and extending to the distal third of the urethra and vagina.

The second case describes an 89 year-old woman undergoing annual checks for scleroatrophic lichen for the last three years who attended before her check because the lesion had grown. On inspection, the lesion covered the left labia minora and majora and lower third of vagina, but the urethra was not affected.

Results

The same entity presents two different onsets, with varying degrees of differentiation. In the first case the biopsy was found to be positive to human papilloma virus protein. This type of lesion is more frequently found in younger patients. The second type of onset usually derives from previous lesions such as lichen and is more frequently found in older patients. Both patients have only recently been diagnosed, therefore therapeutic management cannot be described.

Conclusion

Vulvar cancer is an infrequent disease. It is usually diagnosed at early stages and treatment includes surgical resection. It is important to advise patients to attend in case of dubious findings in the vulvar region, in order to achieve early diagnosis.

References

1. Di Paola G. Cáncer de vulva. Enciclopedia medicoquirúrgica. E- 520-A- 10. 2. Belardi MG, Di Paola GR, Vigh S, Gómez Rueda N. Neoplasia de la vulva y sus enfermedades precursoras. Monografías de dermatolog, 1993; 6, nº4: 25 3. Gómez Rueda N, Garcia A, Belardi M, di Paola GR, Epithelial alterations adjacent to invasive squamous carcinoma of the vulva. J. Repr. Med, 1992; 34: 221.
2. Belardi MG, Di Paola GR, Vigh S, Gómez Rueda N. Neoplasia de la vulva y sus enfermedades precursoras. Monografías de dermatolog, 1993; 6, nº4: 25

P19-03

IMMUNE ACTIVATION ENHANCES EPITHELIAL NERVE GROWTH IN LOCALIZED PROVOKED VULVODYNIA

P. Tommola¹, L. Unkila-Kallio¹, A. Paetau², S. Meri³, E. Kalso⁴, J. Paavonen¹

¹Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (Finland), ²Pathology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (Finland), ³Bacteriology and Immunology, Haartman Institute, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (Finland), ⁴Pain Clinic, Anesthesiology, Intensive Care, and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (Finland)

Background / Objectives

Localized provoked vulvodynia (LPV) manifests with allodynia in the vulvar mucosa. The exact mechanisms resulting in the altered pain sensation are unknown. Generation of the pain may be due to activation of the neuroinflammatory pathways. Thus, we wanted to study whether expression of intraepithelial nerve fibers (IENF) and expression of nerve growth factor (NGF) are related to immune activation in LPV.

Methods

Vestibular mucosal specimens were obtained from 27 patients with severe LPV treated by vestibulectomy and from 15 controls. We employed antibodies against the protein gene product 9.5 (PGP9.5), the neuron specific neurofilament (NF2F11) and nerve growth factor (NGF) for immunohistochemistry to detect IENFs and expression of NGF positive immune cells in the vestibular mucosa. For PGP9.5 positive IENFs we determined the linear density (fiber counts per mm of the outer epithelial surface). NF2F11 positivity was defined as presence or absence of IENFs. NGF was evaluated by counting the staining positive immune cells. Antibodies against CD20 (B lymphocytes) and CD3 (T lymphocytes) were used to identify mucosal areas with increased density of B lymphocytes and the presence of germinal centers, i.e. signs of immune activation. B cell activation index (BAI) was used to describe the overall intensity of B cell infiltration. Mann-Whitney U-test or Wilcoxon signed ranks test and chi²-test or Fischer's exact test were used for statistical analyses.

Results

We found more PGP9.5 positive IENFs in LPV than in controls, $p=0.006$. NF2F11 positive IENFs were found in 17 (63.0%) of 27 LPV cases and in none of the controls. The occurrence of IENFs was more common in samples with more pronounced immune activation. NGF positive immune cells were more numerous in the mucosal areas with high B cell infiltration and IENFs than in the areas without. Glandular epithelium was identified in 14 LPV samples. PGP9.5 positive nerve fibers were found at significantly higher densities in glands surrounded by B cell infiltrations than in glands without B cells, $p=0.013$. Also, NF2F11 fiber positivity in the glandular epithelium was associated with immune activation.

Conclusion

Excessive epithelial nerve growth in LPV is associated with increased B cell infiltration and NGF positivity. This supports the fundamental role of immune activation in LPV.

P20-01

THERAPEUTIC HPV16/18 ADENOVIRUS-VECTOR-BASED VACCINE PROOF OF CONCEPT IN MICE AND NON HUMAN PRIMATES

S. Khan¹, K. Oosterhuis¹, K. Wunderlich¹, E. Bunnik¹, M. Bhaggoe¹, S. Boedhoe¹, S. Karia¹, J. Serroyen¹, S. Janssen¹, J. Vellinga¹, G. Schepe¹, D. Barouch², J. Custers¹, R. Zahn¹

¹Janssen Infectious Diseases and Vaccines, Pharmaceutical Companies of Johnson and Johnson (Netherlands), ²Beth Israel Deaconess Medical Center, Harvard Medical School (United States of America)

Background / Objectives

The viral proteins that are expressed in HPV-infected (pre)malignant cells are considered ideal targets for immunological intervention. Most vaccine approaches for therapeutic vaccination aim to induce of HPV16 E7- and/or E6-specific cellular immunogenicity. As clinical success has so far been limited, novel approaches are required. We present the pre-clinical proof of concept of a replication-deficient adenovirus type 26 and 35 based vaccine for the treatment of HPV16 and HPV18 induced lesions.

Methods

We developed HPV16- and HPV18-specific antigens consisting of a fusion protein of non-functional E2, E6 and E7. By combing these antigens the Adenovirus vector-based vaccine will be suitable for the treatment of both early and late stage lesions, as E2 is predominantly expressed in early stage lesions whereas E6 and E7 are expressed in late stage lesions. Induction of HPV16 and HPV18-specific T-cells were assessed in mice and non-human primates (NHP).immunized with the vaccine. Therapeutic vaccine efficacy was evaluated using the well characterized TC-1 mouse tumor model.

Results

Robust and long-lasting T-cell immunogenicity was induced upon immunization of mice and NHP with adenoviral vectors encoding the designed antigens. The developed vaccine vectors showed higher therapeutic efficacy compared to a benchmark control vaccine consisting of HPV16 E7 derived synthetic long peptides in the TC-1 mouse model.

Conclusion

The favorable immunogenicity and efficacy profile of the adenovirus based vectors along with the established procedures to manufacture these vectors at a large scale makes this approach attractive for clinical evaluation.

References

Khan et al (manuscript under preparation)

EUROGIN 2016 ABSTRACTS

PART IV INDEX OF AUTHORS

A

Abad Rubio C. [OC 12-14](#), [P09-02](#), [P09-03](#)
Abrahamsen M. [SS 12-08](#)
Abramowitz L. [P12-18](#)
Abramson A. [HN 05-01](#)
Adachi S. [WACC II-13](#)
Adalety R. [OC 12-03](#)
Adegboyega O. [P17-05](#)
Adhikari I. [P07-01](#)
Aggarwal C. [Ssim 02-07](#)
Aggelis E. [OC 08-11](#)
Agorastos T. [OC 01-06](#), [OC 01-09](#), [OC 08-02](#), [OC 08-11](#), [OC 10-06](#), [P11-06](#)
Ahmad A. [SS 09-01](#)
Akbari A. [OC 01-04](#)
Akhmedova A. [P18-01](#)
Akiko T. [P12-23](#)
Akira W. [P10-02](#)
Akiyama Y. [P11-01](#)
Akwada G. [OC 12-03](#)
Alahmari S. [WACC II-02](#)
Alamari Z. [WACC II-02](#)
Alan N. [P17-05](#)
Alba A. [P07-04](#)
Alberts C.J. [WACC II-15](#), [WACC II-16](#)
Albieri V. [SS 13-02](#)
Albogami S. [WACC II-02](#)
Albuquerque A. [P12-01](#)
Alemany L. [OC 07-04](#)
Alfaro K. [OC 09-10](#), [SS 02-03](#)
Algazi A. [HN 04-02](#)
Algieri F. [P13-01](#)
Alhamlan F. [OC 05-04](#)
Al-Hussaini A. [P10-03](#)
Aliaga-Lozano F. [P12-02](#)
Ali-Risasi C. [P14-01](#)
Al-Khishali T.J.M. [OC 15-06](#)
Allan W. [OC 12-04](#)
Allison P. [HN 08-03](#)
Almonte M. [P15-03](#)
Alotaibi M. [WACC II-02](#)
Alsarhani W. [WACC II-02](#)
Alvarez M. [OC 04-09](#)

Ambrosch P. [HN 08-06](#)
Amit A. [CS 04-04](#)
Amuah J.E. [OC 12-18](#)
Amuah J. [OC 12-03](#)
Anagnou N.P. [OC 11-10](#), [P15-01](#)
Anagnou N. [OC 11-13](#), [P15-04](#)
Andersen B. [OC 02-07](#), [P17-01](#)
Andersson S. [OC 02-02](#), [OC 12-07](#), [P12-10](#)
Andrae B. [OC 02-03](#), [OC 02-05](#), [P07-08](#), [STC 02 I-05](#), [WACC II-08](#)
Andreassen T. [OC 14-11](#), [WACC II-03](#)
Angelidou S. [OC 08-02](#), [OC 10-06](#)
Angelis E. [OC 08-02](#), [OC 10-06](#)
Anger D. [OC 13-14](#), [P11-09](#)
Anttila A. [OC 14-03](#), [OC 14-05](#), [STC 02 I-01](#)
Anttila A. [MSS 01 A-01](#)
Apter D. [P07-01](#)
Aragón M.A. [OC 08-03](#)
Aragón M.Á. [P15-02](#)
Arakawa I. [WACC II-12](#)
Arbyn M. [CS 03-04](#), [HN 08-05](#), [MSS 02-06](#), [MTC 03 I-07](#), [OC 01-04](#), [OC 04-01](#), [OC 14-04](#), [OC 14-08](#),
[OC 14-13](#), [P03-02](#), [P07-02](#), [SS 14-05](#)
Archibald S. [HN 09-07](#)
Argyri E. [P07-14](#)
Arias M. [HN 09-07](#)
Arjola X. [GS 03-04](#), [OC 14-07](#)
Arnheim-Dahlström L. [OC 05-06](#), [P07-15](#), [WACC II-17](#)
Asai-Sato M. [WACC I-06](#), [WACC II-18](#), [WACC II-21](#)
Asakawa K. [OC 08-08](#)
Asciutto C. [OC 02-02](#)
Ashelford K. [P10-03](#)
Athanasiadis L. [P11-06](#)
Atsushi M. [P10-02](#)
Audirac-Chalifour A. [OC 11-15](#)
Auslander R. [CS 04-04](#)
Austin J. [SS 06-03](#)
Azike J. [OC 07-04](#)

B

Baasland I. [OC 14-06](#)
Badoual C. [HN 07-01](#), [HN 08-08](#)
Bagarrazi M. [Ssim 02-07](#)
Baggio M.L. [OC 07-06](#), [SS 12-08](#)
Bahena-Román M. [OC 11-15](#)

Baillet M.V.P. [P01-06](#)
Baillou C. [HN 07-03](#)
Baio G. [OC 13-14](#)
Baker E. [OC 10-05](#)
Bakker R. [OC 13-06](#)
Balázs P. [WACC II-04](#)
Baldauf J.J. [OC 01-10](#), [P07-02](#)
Balla B.C. [WACC II-04](#)
Ballester Galiana H. [P07-04](#)
Banwait R. [SS 06-03](#)
Baraniuk M.S. [P17-11](#), [SS 05-03](#)
Baraniuk S. [P17-05](#)
Barazandeh M. [OC 15-07](#)
Barbe C. [OC 01-10](#)
Barbosa R.H. [P08-01](#)
Barouch D. [P20-01](#)
Barradeau S. [OC 01-10](#)
Barrios E. [P02-01](#)
Bass S. [MTC 03 II-01](#)
Bassett R. [P12-07](#)
Basten M. [OC 11-06](#)
Basu P. [CS 05-03](#)
Baudin M. [MSS 05-05](#)
Bauml J. [Ssim 02-07](#)
Baussano I. [MSS 05-07](#), [MTC 03 II-03](#)
Bautista O. [OC 13-13](#), [STC 01 A-02](#)
Bawazeer M. [WACC II-02](#)
Baxter G. [OC 12-04](#)
Bay P. [OC 04-06](#), [OC 15-01](#)
Beachler D. [SSim 01-01](#)
Becker S. [OC 09-03](#)
Beckmann M.W. [GS 03-02](#)
Beddows S. [MSS 06 A-02](#)
Beert J. [OC 10-04](#)
Behnke A.L. [OC 12-18](#)
Behr M. [OC 12-06](#)
Beires J. [OC 05-02](#)
Bekkers R. [OC 04-04](#), [OC 12-09](#), [OC 12-10](#)
Belaya J. [P07-03](#), [P11-08](#)
Belfort Mattos P. [P01-09](#)
Belinson J. [MSS 02-06](#)
Bell S. [OC 07-07](#)
Bellardini P. [P12-05](#)
Bellier B. [HN 07-03](#)

Benabderrahmane D. [P12-18](#)
Benchetrit M. [OC 01-10](#)
Benczik M. [OC 11-11](#), [OC 12-13](#)
Benevolo M. [P12-03](#)
Benito A. [P12-09](#)
Bennasar-Figueras A. [P12-02](#)
Bennett P. [OC 11-16](#), [SS 09-05](#)
Benoy I. [MTC 03 I-07](#), [OC 01-04](#), [OC 05-03](#)
Bentley J. [OC 04-04](#)
Beqaj S.S. [OC 10-05](#)
Bergengren L. [P17-02](#)
Bergeron C. [P07-02](#)
Berkhof J. [MSS 03-04](#), [MTC 03 I-07](#), [OC 03-06](#), [OC 04-08](#), [OC 09-07](#), [OC 11-03](#), [OC 11-07](#), [OC 11-17](#),
[OC 12-10](#), [OC 12-15](#), [SS 03-01](#), [SS 08-03](#), [SS 08-06](#), [SS 15-07](#)
Bernardino M. [P11-02](#)
Bernhard A. [OC 11-08](#)
Bernhardt A. [GS 03-04](#), [OC 14-07](#)
Bersani C. [HN 03-01](#)
Bertolus C. [HN 07-03](#)
Bertram A. [OC 10-03](#)
Best M. [SSim 02-06](#)
Bevens T. [P12-07](#)
Beyers K. [OC 12-08](#), [P12-16](#), [P12-17](#), [P12-21](#)
Bhaggoe M. [P20-01](#)
Bhatia R. [CS 01-01](#), [MTC 03 I-10](#), [OC 01-07](#), [SS 11-06](#)
Bi D. [OC 03-05](#), [OC 13-11](#)
Bianic F. [P01-07](#), [P11-09](#)
Biaye B. [P17-03](#)
Bienvenu L. [P12-18](#)
Biesmans S. [P12-16](#), [P12-17](#), [P12-21](#), [P12-22](#)
Bilancioni A. [OC 12-11](#)
Birembaut P. [HN 01-05](#), [HN 08-04](#)
Bissery M.C. [SSim 02-06](#), [Ssim 03-04](#)
Bjørge T. [SS 13-04](#)
Blaakær J. [P17-01](#)
Blaga L. [P17-07](#)
Block S. [OC 13-04](#)
Blumenfeld Z. [CS 04-04](#)
Boa R. [OC 01-08](#)
Böcher O. [OC 01-06](#), [OC 01-09](#)
Boecher O. [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Boedhoe S. [P20-01](#)
Boers A. [OC 11-02](#)
Bogaards J.A. [OC 03-06](#)

Bogers J.P. [MTC 03 I-07](#), [OC 10-04](#), [OC 14-04](#)
Bogers J. [OC 01-04](#), [OC 05-03](#)
Bögershausen B. [HN 08-01](#)
Bohm-Starke N. [STC 01 B-02](#)
Bohr-Mordhorst L. [P01-03](#)
Boland J. [MTC 03 II-01](#), [SS 09-02](#)
Boland E. [OC 01-07](#)
Bolatti E.M. [OC 15-02](#)
Bollaerts K. [OC 06-05](#)
Bonagura V. [HN 05-01](#)
Bonanni P. [OC 06-04](#), [OC 06-05](#), [OC 13-14](#)
Bonde J. [MTC 03 I-07](#), [OC 10-01](#), [OC 12-01](#), [OC 12-02](#), [OC 12-19](#)
Borchgrevink-Pedersen S. [OC 01-03](#)
Borena W. [OC 14-15](#)
Borget I. [HN 08-08](#)
Borgfeldt C. [OC 02-02](#)
Bornstein J. [STC 01 A-01](#), [STC 01 A-06](#)
Börsch C. [GS 03-04](#), [OC 11-08](#)
Bory J.P. [OC 01-10](#)
Bosch X. [OC 06-04](#)
Bosch F.X. [OC 06-05](#), [OC 07-04](#)
Bosch José F.X. [SS 02-01](#), [SS 08-02](#)
Boschetti E. [OC 01-06](#), [OC 01-09](#), [OC 08-11](#)
Boschetti-Grützmaier E. [OC 08-02](#), [OC 10-06](#), [OC 11-06](#)
Bosgraaf R. [OC 12-09](#), [OC 12-10](#)
Bottari F. [OC 10-01](#), [P12-13](#), [P17-06](#)
Bouchard C. [SS 13-03](#)
Bouchard-Fortier A. [SS 13-03](#)
Bouchard-Roy G. [SS 13-03](#)
Bouchilloux S. [OC 01-10](#)
Bougel S. [OC 12-11](#)
Boulagnon-Rombi C. [HN 08-04](#)
Boulianne N. [P11-05](#)
Boullier S. [SSim 02-06](#)
Bousquet-Mélou A. [SSim 02-06](#)
Bouten S. [OC 12-06](#)
Boveri S. [P12-13](#), [P17-06](#)
Boyer J. [Ssim 02-07](#)
Bozat-Emre S. [OC 13-02](#)
Bradshaw C.S. [OC 03-08](#)
Brakenhoff R. [HN 01-02](#)
Brandi L. [P04-03](#)
Bremer R. [OC 09-08](#)
Brentnall A.R. [SS 06-03](#)

Brewer N. [WACC II-05](#), [WACC II-07](#)
Bridonneau P. [SSim 02-06](#)
Briko N. [P07-07](#)
Brisson M. [SS 15-02](#)
Brotherton J. [MSS 03-02](#), [SS 01-05](#)
Brouwer E. [MSS 01 B-02](#), [OC 14-13](#), [OC 14-16](#)
Brown B. [OC 04-11](#)
Brown R. [WACC II-19](#)
Brown D. [OC 03-01](#), [OC 13-04](#)
Brucker S. [OC 09-03](#)
Brun J.L. [OC 01-10](#)
Buchinsky F. [HN 05-02](#)
Bulten J. [OC 12-09](#)
Bulycheva E. [P07-03](#), [P11-08](#)
Bumbu M. [P17-07](#), [WACC II-03](#)
Bunnik E. [P20-01](#)
Burandt E. [OC 07-08](#)
Burchett H.E.D. [OC 13-15](#), [WACC II-11](#)
Burdett L. [MTC 03 II-01](#)
Burk R. [MTC 03 II-01](#), [SS 09-02](#)
Busby-Earle C. [OC 01-05](#), [SS 11-06](#)
Buttmann-Schweiger N. [GS 01-05](#)
Byoung-Gie K. [P01-01](#), [P01-08](#)

C

Caballero P. [P07-04](#)
Cabrera Y. [P15-03](#)
Cacciotti J. [P12-05](#)
Cadman L. [SS 06-03](#)
Cai Y. [OC 11-18](#)
Calaresu E. [OC 05-01](#)
Cameron R. [MTC 03 I-10](#)
Campbell C. [OC 12-05](#)
Campbell S. [OC 01-08](#), [P07-06](#)
Campbell J. [HN 04-02](#)
Camuesco D. [P13-01](#)
Canfell K. [MSS 03-02](#), [MSS 05-08](#), [MTC 02-03](#), [SS 03-08](#), [SS 04-02](#), [SS 15-01](#), [SS 15-08](#)
Cano P. [P12-12](#)
Cappelli M.G. [OC 05-07](#)
Carcopino X. [CS 05-02](#), [OC 04-10](#)
Carestiato F.N. [P08-01](#)
Carlsten-Thor A. [OC 14-09](#)
Carow K. [OC 11-12](#), [P16-01](#)

Carozzi F. [SS 04-07](#), [Ssim 03-03](#)
Carpay M. [MSS 01 B-02](#)
Carpentier A.F. [HN 07-03](#)
Carregosa A. [OC 05-03](#)
Carretero R. [SSim 02-05](#)
Cartwright R. [SS 09-05](#)
Caruana M. [MSS 03-02](#)
Carvalho J.P. [OC 05-05](#)
Carvalho Da Silva R.J. [OC 07-06](#)
Cashat M. [OC 06-11](#)
Casiraghi O. [HN 08-08](#)
Castanon A. [OC 08-09](#), [OC 09-03](#)
Castle P. [MSS 03-02](#), [OC 01-03](#), [OC 04-09](#), [OC 08-05](#), [OC 09-08](#), [OC 09-10](#), [SS 02-03](#), [SS 03-08](#)
Castle P.E. [MTC 03 II-01](#)
Castonguay G. [HN 08-03](#)
Catarino R. [OC 08-07](#), [OC 12-11](#)
Cavalcanti S.M.B. [P08-01](#)
Cazorla E. [P19-02](#)
Ceballos K. [OC 09-06](#)
Ceelen L. [P12-17](#)
Cen D. [OC 11-18](#)
Cerutti R. [HN 09-03](#)
Cha S.D. [P16-03A](#)
Chantanee T. [OC 15-03](#)
Chargin A. [HN 09-02](#), [OC 01-11](#), [OC 08-10](#)
Charpentier C. [P12-18](#)
Chatchotikawong U. [OC 05-08](#), [OC 15-03](#)
Chatzistamatiou K. [OC 01-06](#), [OC 01-09](#), [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Chau N. [HN 04-03](#)
Chel Hun C. [P01-01](#), [P01-08](#)
Chelea I. [P19-02](#)
Chen H.C. [P04-07](#)
Chen J. [OC 13-13](#), [SSim 01-04](#)
Chen M.Y. [OC 03-08](#), [SS 12-07](#)
Chen C.A. [P04-07](#)
Chen Z. [MTC 03 II-01](#)
Cheng Y. [P08-03](#)
Chernesky M. [HN 09-07](#)
Chesson H. [MSS 06 A-01](#)
Chiao E. [P17-11](#), [SS 05-02](#), [SS 05-03](#)
Chiappetta C. [P12-05](#)
Chiaravalli A.M. [HN 09-03](#)
Chikazawa K. [P11-01](#)
Chiu C.H. [OC 06-06](#)

Cho C.H. [P16-03A](#), [SSim 02-04](#)
Cholkeri-Singh A. [OC 04-02](#)
Choly N. [OC 11-06](#)
Chotewutmontri S. [OC 11-12](#)
Chouhy D. [OC 15-02](#)
Chow E.P.F. [OC 03-08](#)
Chow E.P. [SS 12-07](#)
Christensen E.H. [OC 12-19](#)
Christiansen I.K. [OC 01-03](#), [P08-02](#)
Christine C. [OC 02-06](#)
Christoph B. [OC 14-07](#)
Christou P. [P15-01](#)
Chung H. [P16-03A](#)
Ciavardelli D. [P07-16](#)
Cintra R. [OC 07-06](#)
Clark J. [HN 04-03](#)
Clarke R. [P12-04](#)
Clavel C. [HN 08-04](#), [HN 09-04](#), [OC 01-10](#)
Clayburgh D. [HN 04-04](#)
Clemente E. [OC 08-03](#), [P15-02](#)
Clifford G. [HN 09-04](#), [MTC 01-01](#), [SS 09-02](#), [SSim 03-02](#)
Clifford G.M. [MTC 03 II-03](#)
Cochicho D. [OC 07-09](#)
Cochino E. [SS 07-02](#)
Cocuzza C. [OC 05-01](#), [P05-03](#)
Coelho A. [OC 07-09](#), [P11-02](#)
Coen E. [SS 09-04](#)
Cohen D.D. [OC 14-10](#)
Cohen C.R. [OC 08-09](#)
Cohen R. [Ssim 02-07](#)
Cohet N. [SSim 02-06](#)
Coldman A. [OC 08-08](#), [OC 09-06](#)
Coles V. [P01-07](#)
Combes J.D. [HN 09-04](#)
Comes M.D. [OC 08-03](#), [P15-02](#)
Consalvo A. [P07-16](#)
Cook D. [OC 09-06](#)
Cook-Wiens G. [HN 01-03](#)
Cooper C. [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Cornall A.M. [SS 12-07](#)
Cornall A. [P05-02](#)
Corner A. [P16-01](#)
Cortés J. [OC 11-14](#), [P13-01](#)
Costa A.P. [OC 06-07](#)

Cotton S. [CS 01-03](#), [CS 01-04](#)
Coutlée F. [HN 08-03](#)
Couvelard A. [P12-18](#)
Covello R. [P12-03](#)
Coza D. [P17-07](#)
Cremer M. [OC 04-09](#), [OC 09-10](#), [SS 02-03](#)
Cruickshank M. [CS 01-03](#), [CS 01-04](#), [CS 02-03](#), [CS 03-02](#), [MTC 03 I-10](#), [OC 01-05](#)
Cubie H. [CS 01-01](#), [MTC 03 I-03](#), [OC 02-06](#), [OC 12-05](#), [SS 11-06](#)
Cullen M. [MTC 03 II-01](#), [SS 09-02](#)
Cunha M. [OC 07-09](#), [P11-02](#)
Cury L. [OC 14-10](#), [P17-12](#)
Cuschieri K. [CS 01-01](#), [CS 01-02](#), [CS 01-04](#), [MTC 03 I-07](#), [MTC 03 I-10](#), [OC 01-05](#), [OC 01-07](#), [OC 07-07](#), [SS 11-06](#)
Custers J. [P20-01](#)
Cuvelier C. [P16-02](#)
Cuzick J. [MSS 01 A-04](#), [MSS 02-06](#), [MTC 03 I-04](#), [SS 06-03](#), [SS 09-01](#)

D

Dalianis T. [HN 03-01](#), [WACC II-08](#)
Dalstein V. [HN 08-04](#), [HN 08-08](#), [HN 09-04](#), [OC 01-10](#)
Danielewski J.A. [OC 03-08](#)
Dannecker C. [GS 03-02](#)
Dansonka-Mieszkowska A. [P07-11](#)
Darai E. [OC 01-10](#)
Darlington-Brown J. [MSS 03-02](#)
Darragh T. [OC 04-03](#)
Das R. [OC 03-03](#), [OC 13-03](#), [OC 13-04](#)
Davitian C. [P12-18](#)
Dawlett M. [P12-07](#)
De Bruin H. [OC 12-10](#)
De Jongh N. [MSS 01 B-02](#)
De Koeijer M. [OC 12-08](#), [P12-16](#), [P12-17](#), [P12-21](#)
De Kok I. [OC 02-01](#), [OC 14-02](#)
De Kok I.M.C. [OC 02-04](#)
De Kok I.M.C.M. [OC 14-01](#)
De Koning M. [OC 01-01](#), [OC 12-08](#)
De Kuyper-De Ridder G. [OC 12-09](#)
De Martel C. [MTC 01-01](#)
De Melker H.E. [OC 03-04](#)
De Meyer T. [OC 11-02](#)
De Sanjose S. [MTC 01-02](#), [SS 14-02](#), [SSim 03-01](#)
De Sanjosé S. [OC 07-04](#)
De Serres G. [P11-05](#)

De Simoni S. [OC 06-06](#)
De Strooper L. [OC 11-17](#), [OC 12-10](#), [OC 12-15](#)
De Thurah L. [OC 12-02](#)
De Vlas S. [OC 13-06](#)
De Vries H. [WACC II-15](#), [WACC II-16](#)
De Waure C. [OC 13-14](#)
Debus J. [OC 11-05](#)
Deceuninck G. [P11-05](#)
Dede Cohen D. [P17-12](#)
Deebukham P. [OC 13-17](#)
Dehlendorff C. [OC 09-01](#)
Del Pino M. [CS 03-03](#), [OC 11-09](#), [OC 12-08](#)
Deleré Y. [P07-09](#)
Delgado García S. [P07-04](#)
Delgado-Romero K. [OC 11-15](#)
Della Rocca C. [P12-05](#)
Deng Z. [HN 08-07](#)
Denier J.F. [P12-08](#)
Denny L. [OC 01-08](#)
Depardon J. [P12-08](#)
Depuydt C. [MTC 03 I-07](#), [OC 10-04](#)
Derkay C. [HN 05-05](#)
Descamps D. [OC 06-06](#)
Deschamps L. [P12-18](#)
Descombes B. [P12-08](#)
Devi R. [OC 06-10](#)
Devoti J. [HN 05-01](#)
Dexeus D. [OC 11-14](#), [P13-01](#)
D'hauwers K. [OC 13-10](#)
Di Cristofano C. [P12-05](#)
Di Stefano F. [MSS 01 A-05](#)
Di Toro F. [P07-16](#), [P12-25](#)
Dijkstra M. [OC 11-07](#), [OC 12-15](#)
Dijkstra M.G. [OC 09-07](#)
Dillner J. [MSS 05-07](#), [MTC 03 I-05](#), [OC 14-09](#), [SS 08-05](#), [SS 09-06](#), [SS 11-04](#), [STC 02 I-05](#)
Diniz M. [HN 07-01](#)
Dinubile M. [OC 13-04](#)
Dionne M. [OC 06-09](#)
Diouf A. [P04-01](#), [P17-03](#)
Dirix M. [OC 10-04](#)
Dobson S. [OC 06-09](#)
Doerwald-Munoz L. [HN 09-07](#)
Dominguez M.P. [P12-09](#)
Dominiak-Felden G. [OC 13-04](#)

Donà M.G. [P12-03](#)
Donahue K. [WACC II-07](#)
Donders G. [OC 05-02](#)
Donken R. [OC 03-04](#)
Donne C. [P12-08](#)
Doorbar J. [OC 11-09](#)
Dörr A. [P03-01](#)
Doucette S. [OC 04-04](#)
Douvier S. [OC 01-10](#)
Dovedi S. [HN 09-08](#)
Drake T. [OC 04-03](#)
Dransart E. [HN 07-01](#)
Dray-Spira R. [SS 07-03](#)
Drechsler I. [OC 01-06](#)
Dreyer G. [OC 02-08](#)
Drury R. [OC 06-05](#), [OC 13-04](#)
D'souza G. [HN 02-01](#)
Du W. [OC 11-18](#)
Duensing S. [OC 11-04](#)
Duff S. [Ssim 02-07](#)
Duk-Soo B. [P01-01](#), [P01-08](#)
Dunyo P. [OC 12-03](#), [OC 12-18](#)
Durando P. [OC 06-06](#)
Dürst M. [OC 11-01](#), [OC 11-12](#), [P16-01](#)
Duvivier M. [P10-01](#)
Dvorak V. [P09-01](#)
Dwyer M. [HN 04-02](#)
Dzhidzhikhia L. [P11-08](#)

E

Ebisch R. [OC 12-09](#)
Eckert K. [OC 09-05](#)
Egawa-Takata T. [WACC I-06](#)
Ehsan I. [HN 07-02](#)
Eijssink J.J.H. [OC 12-16](#)
Ejegod D. [OC 12-02](#)
Ejegod D.M. [OC 10-01](#), [OC 12-01](#), [OC 12-19](#)
Eklund C. [OC 14-09](#), [SS 09-06](#)
Elfstrom K.M. [OC 02-05](#)
Elfström M. [MSS 01 A-02](#), [MSS 05-07](#), [OC 14-09](#), [P07-08](#), [SS 08-05](#), [SS 09-06](#), [STC 02 I-05](#)
Elfström K.M. [OC 02-03](#)
Elizabeth C. [P17-05](#)
Ellis K. [OC 10-02](#)

Eluf-Neto J. [OC 14-10](#), [P17-12](#)
El-Zein M. [OC 12-06](#), [P02-01](#)
Endel G. [OC 14-15](#)
Engesæter B. [OC 01-03](#), [OC 14-11](#), [OC 14-12](#)
Engsæther B. [OC 08-06](#)
Enomoto T. [WACC I-06](#), [WACC II-13](#), [WACC II-18](#)
Esposito S. [OC 06-06](#)
Esquerré M. [SSim 02-06](#)
Esteban E. [OC 14-15](#)
Eulenburg C. [OC 07-08](#)
Evans M. [P10-03](#)

F

Faber M. [OC 14-07](#)
Faber M.T. [SS 13-02](#)
Fabian M. [P03-01](#)
Facco C. [HN 09-03](#)
Fadhilah F. [SS 09-07](#)
Fairley C.K. [OC 03-08](#), [SS 12-07](#)
Fait T. [P09-01](#)
Farias K.J. [OC 06-07](#)
Faryna J. [P07-11](#)
Faye Dieme M.E. [P04-01](#)
Faye Diémé M.E. [P17-03](#)
Fehler G. [OC 03-08](#)
Fehm T. [P04-03](#)
Fehr M. [GS 03-02](#)
Fehrmann S. [OC 01-06](#), [OC 01-09](#)
Feletto M. [OC 13-15](#), [WACC II-11](#)
Felix A. [OC 07-09](#)
Felix J. [OC 04-09](#), [OC 09-10](#), [SS 02-03](#)
Fender M. [P07-02](#)
Fennema H. [WACC II-15](#), [WACC II-16](#)
Fernandes S.M. [P04-06](#)
Ferrera A. [P15-03](#)
Fetterman B. [OC 08-05](#), [OC 09-08](#)
Fiander A. [P10-03](#)
Fickle D. [WACC II-20](#)
Findri Gustek S. [P15-05](#)
Fischer M. [OC 14-08](#)
Fisher W. [SS 12-06](#)
Fisher A. [P11-04](#)
Fisher J. [WACC II-20](#)

Flanagan B. [OC 08-08](#)
Fleischhauer M. [OC 01-06](#), [OC 01-09](#)
Florjancic M. [OC 14-17](#)
Focchi G. [P01-09](#)
Folschweiller N. [OC 03-02](#), [OC 06-06](#)
Fong J. [OC 06-10](#)
Fortier M. [SS 13-03](#)
Fortunato F. [OC 05-07](#)
Franceschi S. [HN 09-04](#), [MSS 05-07](#), [MTC 01-01](#), [MTC 03 II-03](#), [SS 03-05](#), [SS 09-02](#)
Franco E. [OC 09-06](#), [OC 12-06](#), [P02-01](#), [SS 03-02](#)
Franco E.L. [HN 08-03](#), [MTC 02-06](#), [SS 06-01](#)
Frazer I. [OC 06-10](#)
Frederiksen M.E. [P01-06](#)
Frederiksen K. [OC 09-01](#), [SS 12-02](#)
Frenette L. [OC 06-06](#)
Friebe K. [OC 04-07](#)
Friedrich M. [GS 03-02](#)
Friel D. [OC 06-06](#)
Fu G. [OC 01-07](#), [P12-21](#)
Fujita H. [P12-20](#), [P12-23](#)
Fulp W. [SS 12-08](#)
Furlan D. [HN 09-03](#)
Fuza L.M.S. [OC 14-10](#)
Fuza L.M. [P17-12](#)

G

Gage J. [MSS 02-06](#), [OC 04-09](#), [OC 08-05](#), [OC 09-10](#), [SS 02-03](#)
Gagnon J. [P01-07](#)
Galaj A. [OC 03-02](#)
Galan L. [OC 07-06](#)
Gale N. [HN 08-02](#)
Gallagher K.E. [OC 13-15](#), [WACC II-11](#)
Gallego I. [P12-12](#)
Galletta H. [HN 09-08](#)
Gálvez J. [P13-01](#)
Ganss L. [OC 11-04](#)
García D. [WACC I-02](#)
García Calderon M. [OC 12-14](#)
García Mollinedo M. [OC 06-11](#)
García Ponz L. [OC 12-14](#)
García-Andrade C. [P12-12](#)
García-Carrancá A. [OC 11-15](#)
Garland S. [OC 13-04](#), [OC 13-13](#), [P05-02](#), [SS 02-04](#), [SS 13-06](#), [SS 13-07](#), [STC 01 A-02](#), [WACC II-18](#)

Garland S.M. [OC 03-08](#), [OC 06-10](#), [SS 12-07](#)
Garrido-Mesa J. [P13-01](#)
Gassama O. [P04-01](#), [P17-03](#)
Gauvreau C. [OC 08-08](#)
Gavin S. [OC 09-06](#)
Gebhardt M. [GS 03-02](#), [HN 08-01](#)
Gebski V. [MSS 03-02](#)
Gedzah I. [OC 12-03](#)
Geng L. [P08-03](#)
Genta M.L.N.D. [OC 05-05](#)
Geraets D. [MTC 03 I-07](#)
Gertig D. [MSS 03-02](#)
Getzin L. [HN 08-01](#)
Geurts-Giele W.R. [OC 15-04](#)
Ghaemmaghaminori F. [P19-01](#)
Gheit T. [HN 09-04](#), [MTC 03 I-07](#), [MTC 03 II-03](#)
Ghelardi A. [OC 04-06](#), [OC 15-01](#)
Ghosh S. [OC 07-05](#)
Giaquinto C. [OC 06-06](#)
Gilbert L. [OC 12-06](#)
Gilca V. [OC 06-09](#), [P11-05](#)
Gilchrist J. [HN 09-07](#)
Gil-De Miguel A. [P07-05](#)
Giles P. [P10-03](#)
Gilham C. [OC 09-09](#), [SS 09-01](#)
Gil-Prieto R. [P07-05](#)
Giraldo P.C. [OC 06-07](#)
Giri A.A. [OC 15-02](#)
Giuliano A.R. [OC 07-06](#)
Giuliano A. [OC 13-13](#), [P07-17](#), [SS 12-08](#), [STC 01 A-02](#)
Goedemans R. [HN 07-02](#)
Gois Speck N. [P01-09](#)
Goldberg S.K. [WACC II-23](#)
Gomes A. [OC 07-09](#)
Gonçalves A.K. [OC 06-07](#)
Gondara L. [OC 09-06](#)
Gondry J. [OC 04-10](#)
Gong Y. [P12-07](#)
Gonindard C. [SSim 02-06](#)
Gonzalez C. [OC 13-16](#)
Goodman M. [HN 01-03](#), [HN 09-01](#), [SS 10-02](#)
Gorelik A. [SS 13-06](#)
Gorka M. [OC 08-03](#)
Görögh T. [HN 08-01](#), [HN 08-06](#)

Gotlieb W. [OC 12-06](#)
Gottvall M. [WACC II-06](#)
Grabe N. [SSim 01-06](#)
Graesslin O. [OC 01-10](#)
Graf A. [P12-14A](#)
Graf A.H. [P12-14A](#), [P12-15B](#)
Graham C. [OC 01-07](#)
Graham S. [OC 07-07](#)
Grandahl M. [WACC II-06](#), [WACC II-08](#)
Grant L. [OC 02-06](#)
Gravitt P. [MTC 01-04](#), [MTC 03 II-07](#), [SS 10-04](#)
Graziosi P.G.C.M. [OC 11-03](#)
Graziosi G.C.M. [OC 04-08](#)
Graziosi P. [OC 11-07](#), [OC 12-15](#)
Greiff L. [OC 02-02](#)
Greinke C. [OC 11-01](#)
Grenman S. [OC 07-03](#), [SS 12-03](#)
Gribble S. [OC 08-08](#)
Griesser H. [GS 03-01](#)
Griffin H. [OC 11-09](#)
Griffiths U.K. [OC 13-15](#), [WACC II-11](#)
Grimm D. [OC 07-08](#)
Grimm C. [GS 03-02](#)
Gröning A. [OC 10-03](#)
Guardia L. [P15-02](#)
Guarin D. [OC 06-11](#)
Guilhem O. [P12-06](#)
Guillot C. [OC 08-07](#)
Guillot-Delost M. [HN 07-03](#)
Guimera N. [OC 07-04](#)
Gulmini C. [P17-06](#)
Gultekin M. [OC 09-04](#)
Guo M. [P12-07](#)
Gupta M. [HN 09-07](#)
Gupta D. [HN 09-02](#)

H

Hackl M. [OC 14-15](#)
Haddad R. [HN 04-03](#)
Haedicke J. [P07-13](#)
Haegblom L. [HN 03-01](#)
Haeringer N. [OC 07-08](#)
Häfner N. [P16-01](#)

Hagemann I. [OC 01-06](#), [OC 01-09](#), [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Håkansson U. [OC 02-02](#)
Halec G. [OC 07-04](#)
Hall M. [SS 04-02](#)
Hallas J. [P01-06](#)
Haller H. [P17-09](#)
Hammer Bech B. [P17-01](#)
Hämmerling G.J. [SSim 02-05](#)
Hammerman P. [HN 04-03](#)
HAMPL M. [GS 04-04](#), [P04-03](#)
Hanley S.J.B. [P12-23](#)
Hanley S. [P12-20](#), [WACC I-04](#), [WACC I-06](#)
Hansel A. [OC 11-01](#)
Hansen B.T. [P07-06](#), [P07-15](#)
Hansen M. [OC 01-03](#), [P08-02](#)
Hansen B. [OC 12-03](#)
Haque J. [HN 04-02](#)
Harder T. [P07-09](#)
Haugland H.K. [SS 13-04](#)
Havanond P. [OC 02-09](#), [OC 08-04](#)
Havanond N. [OC 13-17](#)
Hazenberg E. [OC 15-04](#)
Healey J. [OC 04-11](#)
Heard I. [MTC 03 I-07](#)
Heather C. [OC 12-04](#)
Heaton C. [HN 04-02](#)
Hebette G. [OC 14-08](#)
Hedderich J. [HN 08-06](#)
Heddini U. [STC 01 B-02](#)
Heffernan M. [WACC II-09](#)
Hegarty S. [P12-04](#)
Heideman D.A.M. [OC 02-08](#), [OC 04-08](#), [OC 11-03](#)
Heideman D. [MTC 03 II-05](#), [OC 11-07](#), [OC 11-17](#), [OC 12-09](#), [OC 12-10](#), [OC 12-15](#)
Heijman T. [WACC II-15](#), [WACC II-16](#)
Heinävaara S. [OC 14-03](#), [OC 14-05](#)
Helenius G. [P01-03](#), [P17-02](#)
Heley S. [MSS 03-02](#)
Helmerhorst T.J.M. [OC 04-08](#), [OC 09-07](#), [OC 11-03](#)
Helmerhorst T. [OC 11-07](#), [OC 12-15](#)
Hemmings R. [OC 12-06](#)
Hempel M.H. [OC 01-02](#)
Henes M. [OC 09-03](#)
Hernandez M.L. [P15-03](#)
Hernández R. [P02-01](#)

Hernandez Aragon M. [OC 12-14](#)
Hernández Aragón M. [P09-02](#), [P09-03](#)
Herrero R. [P15-03](#)
Herweijer E. [OC 05-06](#)
Hesselink A. [OC 11-17](#), [OC 12-10](#)
Hezareh M. [OC 06-06](#)
Hicks A. [WACC I-07](#)
Hicks J. [P17-11](#)
Hicks J.T. [SS 05-03](#)
Hildesheim* A. [OC 03-05](#)
Hillemanns P. [GS 03-02](#), [OC 04-07](#), [OC 06-06](#)
Hirakawa H. [HN 08-07](#)
Ho A. [HN 01-03](#)
Ho D. [OC 04-03](#)
Hocking J.S. [SS 12-07](#)
Hoebee B. [MSS 01 B-02](#)
Hoffmann A.S. [HN 08-01](#)
Hoffmann M. [HN 08-01](#), [HN 08-06](#)
Höfler D.H. [OC 01-02](#)
Hogewoning A. [WACC II-15](#), [WACC II-16](#)
Holroyd E. [WACC II-09](#)
Holtzer-Goor K. [OC 14-13](#)
Holzinger D.H. [OC 01-02](#)
Hong S.R. [SSim 02-04](#)
Hontelez J. [OC 13-06](#)
Hopkins M. [CS 01-01](#)
Horn M. [OC 06-06](#)
Hosfield E. [OC 09-08](#)
Hošnjak L. [HN 08-02](#), [OC 15-02](#)
Hotta D. [P11-01](#), [WACC II-12](#)
Howard N. [OC 13-15](#), [WACC II-11](#)
Howie S. [SS 11-06](#)
Hsu H.C. [P04-07](#)
Huang S.H. [HN 02-05](#)
Huang L.M. [OC 06-06](#)
Huijsmans C.J. [OC 15-04](#)
Hultman C. [HN 04-02](#)
Humphris J. [OC 07-02](#)
Hur S.Y. [SSim 02-04](#)
Hutse V. [MTC 03 I-07](#)
Hwang L.Y. [P17-05](#), [P17-11](#), [SS 05-03](#)
Hwang L. [OC 04-03](#)
Hwang Y.Y. [SSim 02-04](#)
Hyklová P. [P03-01](#)

I

Ieven M. [P12-22](#)
Iftner T. [GS 03-02](#), [GS 03-03](#), [OC 09-01](#), [OC 09-03](#), [OC 13-08](#), [P07-13](#), [SS 12-09](#)
Iftner A. [OC 13-08](#), [SS 12-09](#)
Igidbashian S. [P12-13](#), [P17-06](#)
Igor J. [OC 04-13](#)
Ikegami T. [HN 08-07](#)
Ikenberg H. [GS 03-02](#), [GS 03-04](#), [OC 11-08](#), [OC 14-07](#)
Ilona Z. [GS 03-04](#)
Improve Study Group I. [SS 08-03](#)
Ingles D. [SS 12-08](#)
Inthout J. [OC 12-09](#)
Isaeva D. [P07-07](#)
Ito M. [OC 12-17](#)
Itoda I. [P18-02](#)
Itu A. [P17-07](#), [WACC II-03](#)
Ivanus U. [OC 14-17](#)
Ivanuš U. [P17-04](#)
Iversen O.E. [OC 06-03](#), [OC 13-03](#), [SS 13-04](#)
Iwamoto H. [P04-05](#), [P18-02](#)
Iwanari O. [OC 12-17](#)

J

Jackson B. [HN 09-07](#)
Jacob J.A. [P11-09](#)
Jacob J. [P01-07](#)
Jacquet A. [OC 01-10](#)
Jaén-Luchoro D. [P12-02](#)
Jakobsen E. [WACC II-10](#)
Jancar N. [OC 04-12](#)
Jane K. [SS 02-03](#)
Jang D. [HN 09-07](#)
Janik B. [P07-11](#)
Jansen E.E.L. [OC 02-04](#), [OC 14-01](#)
Jansen E. [OC 02-01](#), [OC 14-02](#)
Jansen-Duerr P. [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Jansen-Dürr P. [OC 01-09](#)
Janssen E.A. [OC 01-03](#)
Janssen S. [P20-01](#)
Járay B. [OC 11-11](#), [OC 12-13](#)
Järvelin M.R. [SS 09-05](#)

Jayasinghe Y. [SS 13-06](#)
Jenkins D. [CS 03-03](#), [OC 07-04](#), [OC 11-09](#)
Jenkins G. [SSim 01-01](#)
Jennett C. [MSS 03-02](#)
Jensen P.T. [P01-06](#)
Jensen S.M. [SS 12-02](#)
Jentschke M. [GS 03-02](#), [OC 04-07](#)
Jeon C. [HN 01-03](#)
Jeong-Won L. [P01-01](#), [P01-08](#)
Jermakova I. [OC 04-05](#)
Jerman T. [OC 14-17](#), [P17-04](#)
Jeronimo J. [OC 04-09](#)
Jiang P. [OC 11-18](#)
Jin H.T. [SSim 02-04](#)
Jin F. [HN 04-01](#)
Jit M. [SS 03-06](#), [SS 15-03](#), [SS 15-04](#)
Jo M. [OC 13-05](#)
Johannes L. [HN 07-01](#)
Jorge A.F. [OC 07-09](#), [P11-02](#)
Joseph H. [P17-05](#)
Joura E. [GS 02-02](#), [MSS 05-02](#), [MTC 02-01](#), [OC 06-02](#), [OC 06-04](#), [OC 06-05](#), [OC 13-12](#), [OC 13-13](#), [STC 01 A-02](#)
Judlin P. [OC 01-10](#)
Jung P.S. [P01-04](#)
Junge J. [OC 09-01](#), [SS 13-02](#)
Juras J. [P17-09](#)
Juul K.E. [P07-15](#)

K

Kabakama S. [OC 13-15](#), [WACC II-11](#)
Kabota B. [OC 02-06](#), [OC 12-05](#)
Kado J. [OC 06-10](#)
Kafwafwa S. [OC 02-06](#)
Kai Y. [MTC 03 II-01](#)
Kakizoe T. [WACC II-12](#)
Kaldor J.M. [SS 12-07](#)
Kaliff M. [P01-03](#), [P17-02](#)
Kalliala I. [OC 04-01](#), [OC 11-16](#), [SS 09-05](#)
Kalmar J. [OC 13-07](#), [OC 13-09](#)
Kalso E. [P19-03](#)
Kalteis M.S. [HN 09-05](#)
Kama M. [OC 06-10](#)
Kang C.M. [SSim 02-04](#)

Kang E.S. [P01-02](#), [P04-02](#)
Kang S. [OC 13-05](#)
Kantathavorn N. [OC 05-08](#), [OC 12-12](#), [OC 15-03](#)
Kantelhardt E.J.K. [OC 01-02](#)
Kapadia D. [OC 01-07](#), [P12-21](#)
Karaki S. [HN 07-01](#)
Karakitsos P. [OC 08-10](#)
Karelovic D. [P17-09](#)
Karia S. [P20-01](#)
Karlsson M.G. [P01-03](#), [P17-02](#)
Kasius A. [CS 03-03](#), [OC 11-09](#)
Kasler M. [OC 13-07](#), [OC 13-09](#)
Kasting M. [WACC II-07](#)
Kate C. [OC 12-04](#)
Katsiki E. [OC 08-02](#), [OC 10-06](#)
Kaufmann A.M. [GS 04-01](#), [OC 01-06](#), [OC 01-09](#), [OC 03-02](#), [OC 08-11](#), [OC 11-06](#), [OC 12-03](#), [OC 12-18](#)
Kaufmann A. [OC 08-02](#), [OC 10-06](#)
Kaufmann A.M.K. [OC 01-02](#)
Kavanagh K. [CS 01-01](#), [CS 01-03](#), [CS 01-04](#), [MTC 03 I-10](#), [OC 01-05](#), [OC 07-07](#)
Kavteladze L. [P11-08](#)
Kawaguchi S. [P04-05](#), [P18-02](#)
Kawonga E. [OC 12-05](#)
Kazuo S. [P12-23](#)
Kazuyoshi N. [P10-02](#)
Kazuyoshi S. [P10-02](#)
Keilholz U. [HN 09-05](#)
Keller H. [SSim 02-06](#)
Kellner M. [OC 01-06](#), [OC 01-09](#)
Kemmeren J.M. [OC 03-04](#)
Kempers J.P.P. [OC 02-10](#)
Kero K. [SS 12-03](#)
Kerr S. [SS 12-05](#)
Keschjan L. [P11-08](#)
Khalidin A. [P07-07](#)
Khan S. [P20-01](#)
Khan M. [MSS 02-06](#)
Khanna A. [P12-07](#)
Kharit S. [P07-07](#)
Khemapech N. [OC 02-09](#), [OC 08-04](#), [OC 13-17](#)
Kieninger D. [MSS 05-05](#)
Kikawa S. [P12-23](#)
Killen J. [SS 04-02](#)
Kim H.J. [P01-02](#), [P04-02](#)
Kim J. [OC 09-10](#), [SS 15-05](#), [SS 15-09](#)

Kim C. [WACC II-23](#)
Kim D.Y. [P01-04](#)
Kim S. [OC 13-05](#)
Kim J.H. [P01-02](#), [P04-02](#)
Kim T.J. [SSim 02-04](#)
Kingkaew W. [SS 12-05](#)
Kinney W. [MTC 03 I-09](#), [OC 08-05](#), [OC 09-08](#)
Kirstedter A. [P12-14A](#), [P12-15B](#)
Kitchener H. [OC 09-09](#), [SS 03-08](#)
Kjaer S. [OC 13-04](#)
Kjær S.K. [SS 12-02](#), [SS 13-02](#)
Kjaer S.K. [MSS 06 A-03](#), [OC 09-01](#), [OC 12-01](#), [OC 12-19](#), [P07-15](#)
Kleppe S.N. [P07-08](#)
Kliewer E. [OC 13-02](#)
Kloboves Prevodnik V. [P17-04](#)
Klonos E. [P02-02](#)
Kloor M. [SSim 01-06](#)
Klos U. [OC 11-12](#)
Klug S. [GS 03-02](#)
Koch I. [OC 01-06](#), [OC 01-09](#), [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Kocjan B.J. [HN 08-02](#), [OC 15-02](#)
Kocken M. [OC 11-03](#)
Kocsis A. [OC 11-11](#), [OC 12-13](#)
Kodsi S. [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Koerber S.A. [OC 11-05](#)
Kohut T. [SS 12-06](#)
Koiss R. [OC 11-11](#), [OC 12-13](#)
Komp J.K. [OC 01-02](#)
Konishi H. [OC 12-17](#), [WACC II-12](#)
Konno R. [P11-01](#), [WACC I-06](#), [WACC II-12](#)
Kontostathi G. [OC 11-10](#), [P15-01](#), [P15-04](#)
Konya J. [OC 01-01](#)
Kosalaraksa P. [OC 13-12](#)
Kovacevic D. [P04-04](#)
Kozák M. [P03-01](#)
Krajden M. [OC 06-09](#), [OC 09-06](#)
Krajokkaew P. [OC 05-08](#)
Krasnopolskij V. [P07-03](#)
Krasnopolskiy V. [P11-08](#)
Kraynyak K. [Ssim 02-07](#)
Kraywinkel K. [GS 01-05](#)
Kreimer A.R. [MSS 05-06](#), [MTC 02-04](#), [SS 03-03](#)
Kreimer A. [SSim 01-01](#)
Kremer W.W. [OC 02-08](#)

Krings A. [OC 08-02](#), [OC 10-06](#), [OC 12-03](#), [OC 12-18](#)
Krings A.K. [OC 01-02](#)
Krisorakun W. [OC 05-08](#)
Kristiansen-Haugland H. [P08-02](#)
Krivak Bolanca I. [P15-05](#)
Krongthong W. [OC 05-08](#), [OC 12-12](#)
Kroupis C. [OC 01-01](#)
Krüger Kjaer S. [OC 06-04](#)
Kruger-Kjaer S. [OC 06-05](#)
Kucukyildiz I. [OC 09-04](#)
Kudo R. [WACC I-06](#), [WACC II-13](#)
Kuhn L. [OC 01-08](#)
Kulasingam S. [MSS 03-05](#)
Kulkarni A.S. [OC 03-01](#), [OC 06-11](#), [OC 13-17](#)
Kulkarni A. [OC 13-05](#), [OC 13-07](#), [OC 13-09](#), [P11-03](#), [WACC II-07](#)
Kuster M. [OC 14-17](#)
Kuter B. [OC 13-04](#)
Kwon S.H. [P16-03A](#), [P16-03B](#)
Kwon H.J. [P01-02](#), [P04-02](#)
Kyrgiou M. [CS 04-05](#), [OC 04-01](#), [SS 09-05](#), [SS 10-03](#)

L

Labadie M. [P12-08](#)
Lacau St Guily J. [HN 09-04](#)
Lacau St Guly J. [HN 08-08](#)
Lafalla Bernad O. [P09-02](#), [P09-03](#)
Lagheden C. [SS 09-06](#)
Lahrman B. [SSim 01-06](#)
Lai H.C. [OC 11-11](#)
Lai J. [OC 11-16](#)
Lam J.U.H. [OC 12-01](#), [OC 12-02](#), [OC 12-19](#)
Lamin H. [OC 14-09](#), [SS 09-06](#)
Lamontagne D.S. [OC 13-15](#), [WACC II-11](#)
Landoni F. [P12-13](#), [P17-06](#)
Landy R. [OC 08-09](#)
Laprise C. [HN 08-03](#)
Larger N. [P01-07](#), [P11-09](#)
Larsen M.B. [OC 02-07](#)
Larsson M. [WACC II-08](#)
Latsuzbaia A. [OC 14-08](#)
Lattanzio R. [P07-16](#)
Lau K.W. [SS 09-01](#)
Lauro-Colleaux C. [P12-08](#)

Lavie O. [CS 04-04](#)
Lazcano-Ponce E. [OC 07-06](#), [P02-01](#), [P07-17](#), [SS 12-08](#)
Lazzarato F. [MSS 05-07](#)
Le M. [HN 04-02](#)
Le Moignic A. [HN 07-03](#)
Lecuru F. [OC 01-10](#)
Lee N. [HN 01-03](#)
Lee H.C. [OC 07-02](#)
Lee E.B. [P16-03A](#)
Lee A. [P12-19](#)
Lee M. [OC 09-06](#)
Lee Y.K. [P12-11](#)
Lee J. [Ssim 02-07](#)
Lee J.K. [SSim 02-04](#)
Leeijen C. [OC 15-04](#)
Leeman A. [CS 03-03](#), [OC 11-09](#), [OC 12-08](#)
Lefevre A. [P06-01](#)
Legner S. [P12-14B](#)
Lehtinen M. [P07-01](#), [SS 08-04](#)
Lehtinen* M. [OC 03-05](#)
Lehtinen, On Behalf Of The Hpv-040 Study Group M. [OC 03-07](#)
Lehy T. [P12-18](#)
Lei J. [P07-08](#)
Leinonen M.K. [MSS 01 A-03](#)
Lekka I. [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Lemée F. [OC 01-10](#)
Lemoine F.M. [HN 07-03](#)
Lendaro E. [P12-05](#)
León L. [P02-01](#)
Lescaille G. [HN 07-03](#)
Lesin J. [P17-09](#)
Leveque J. [OC 01-10](#)
Levi J.E. [OC 05-05](#), [OC 14-10](#), [P17-12](#)
Lew J.B. [SS 03-08](#), [SS 04-02](#)
Leybaert K. [P16-02](#)
Li F. [OC 15-05](#)
Li Z. [P08-03](#)
Liaw K.L. [P07-15](#)
Licciardi P.V. [OC 06-10](#)
Lillsunde Larsson G. [P01-03](#), [P17-02](#)
Lima-Silva J. [OC 05-02](#)
Lin J.J. [P04-07](#)
Lin L. [OC 03-02](#)
Lina-Villa L. [P17-12](#)

Lindsay B. [OC 13-04](#), [P11-03](#)
Ljubin-Sternak S. [P07-10](#)
Lockwood G. [OC 08-08](#)
Loenenbach A. [P07-09](#)
Longatto-Filho A. [OC 14-10](#), [P17-12](#)
Löning T. [GS 03-02](#)
Lönnerberg S. [OC 01-03](#), [OC 14-06](#), [OC 14-11](#)
Loopik D. [OC 04-04](#)
Lopalco P.L. [MSS 04-01](#)
Lopez R.M. [OC 05-05](#)
Lopez N. [P07-05](#)
Lopukhov P. [P07-07](#)
Lorena G. [OC 08-03](#)
Lorey T. [MTC 03 II-01](#), [OC 08-05](#), [OC 09-08](#)
Lorincz A. [SS 09-01](#)
Lorincz A.T. [MSS 02-04](#), [MTC 03 II-04](#), [SS 06-03](#)
Losa F. [OC 11-14](#)
Louvanto K. [OC 07-03](#), [OC 12-06](#)
Lucan P. [P03-01](#)
Lucs A. [HN 05-01](#)
Luna Casas G. [OC 06-11](#)
Luostarinen T. [P07-01](#), [SS 08-04](#)
Lupashko O. [P07-07](#)
Luttmer R. [OC 11-03](#), [OC 11-07](#), [OC 12-15](#)
Lutz M. [OC 06-11](#)
Luxembourg A. [OC 03-01](#), [OC 06-01](#), [OC 06-08](#), [OC 13-12](#), [OC 13-13](#), [P11-04](#), [STC 01 A-02](#)
Luyten A. [OC 13-08](#), [P05-01](#), [SS 12-09](#)
Lydrup M.L. [OC 02-02](#)
Lygirou V. [OC 11-10](#), [P15-01](#), [P15-04](#)
Lynge E. [MSS 03-06](#), [OC 12-01](#), [OC 12-02](#), [OC 12-19](#), [P01-06](#), [P17-08](#)
Lyon R. [OC 04-11](#)
Lyons D. [OC 11-16](#)
Lytwyn A. [HN 09-07](#)

M

Ma W. [SSim 01-05](#)
Macdonald M. [WACC II-23](#)
Macedo R. [HN 07-03](#)
Machado C.L. [P08-01](#)
Machado P.R. [OC 06-07](#)
Machalek D. [OC 03-08](#), [P05-02](#)
Machalek D.A. [SS 12-07](#)
Madathil S.A. [HN 08-03](#)

Madrid-Marina V. [OC 11-15](#)
Maeda H. [HN 08-07](#)
Maggino T. [CS 06-01](#)
Maglaveras N. [OC 08-02](#), [OC 10-06](#)
Mahmud S. [OC 13-02](#)
Mahner S. [OC 07-08](#)
Maier T. [WACC II-14](#)
Maier C. [WACC II-14](#)
Maisonneuve D. [P12-08](#)
Makkonen P. [OC 14-03](#)
Makridakis M. [P15-01](#), [P15-04](#)
Malaník J. [P03-01](#)
Manchanda R. [CS 06-04](#)
Mancini J. [OC 04-10](#)
Mangrolia D. [Ssim 02-07](#)
Mangseth Staby K. [OC 01-03](#)
Marc A. [OC 12-04](#)
Maria K. [OC 11-16](#)
Marijan T. [P07-10](#)
Marketos C. [P07-14](#)
Markowitz L. [MSS 06 A-01](#)
Marlowe E. [OC 09-08](#)
Marra E. [WACC II-15](#), [WACC II-16](#)
Martin J. [OC 13-12](#)
Martin R. [OC 09-06](#)
Martín Bayón T.A. [P07-04](#)
Martinelli M. [OC 05-01](#), [P05-03](#)
Martinelli D. [OC 05-07](#)
Martínez Escoriza J.C. [P07-04](#)
Martínez-Barnetche J. [OC 11-15](#)
Martin-Hirsch P. [OC 04-01](#)
Martinková M. [P03-01](#)
Martins T.R. [OC 05-05](#), [OC 14-10](#), [P17-12](#)
Martins L. [OC 07-09](#)
Martus P. [P07-13](#)
Masayoshi S. [P10-02](#)
Massuger L. [OC 12-09](#), [OC 12-10](#)
Mateo V. [HN 07-03](#)
Mateos M.L. [P12-09](#)
Mathewos A.M. [OC 01-02](#)
Matsuyama Y. [OC 12-17](#)
Matthijsse S.M. [OC 02-04](#), [OC 14-01](#)
Matthijsse S. [OC 02-01](#), [OC 13-06](#), [OC 14-02](#)
Mautanga M. [OC 12-05](#)

Maza M. [OC 04-09](#), [OC 09-10](#), [SS 02-03](#)
Mcnamara S. [OC 01-06](#)
Mcneil S. [OC 06-06](#), [OC 06-09](#)
Meadows B. [P11-10](#)
Medina D. [OC 06-11](#)
Meijer C. [MSS 05-05](#), [MTC 03 I-07](#), [OC 06-04](#), [OC 06-05](#), [OC 11-07](#), [OC 11-17](#), [OC 12-09](#), [OC 12-10](#),
[OC 12-15](#), [SS 03-01](#), [SS 08-06](#)
Meijer C.J.L. [OC 02-08](#), [OC 04-08](#), [OC 09-07](#)
Meijer C.J.L.M. [OC 11-03](#)
Meisal R. [P08-02](#)
Melchers W. [OC 12-09](#), [OC 12-10](#)
Memon S. [OC 08-08](#)
Mennini F.S. [OC 13-14](#)
Meredith Y. [MTC 03 II-01](#)
Meri S. [P19-03](#)
Merikukka M. [OC 03-05](#)
Mermelekas G. [P15-01](#)
Merrett N. [OC 07-02](#)
Merz I. [HN 08-06](#)
Messina J. [SS 12-08](#)
Mestrovic T. [P04-04](#)
Meunier F. [P11-04](#)
Meyer-Hamme U. [OC 12-11](#)
Michael R. [P17-05](#)
Micheletti L. [STC 01 A-06](#)
Migliore G. [P12-05](#)
Mihevc Ponikvar B. [OC 04-12](#)
Mikio N. [P10-02](#)
Milbourne A. [P12-07](#)
Millan D. [OC 07-07](#)
Millas S. [SS 05-03](#)
Miller A. [OC 08-08](#)
Minst M. [P12-10](#)
Mints M. [HN 03-01](#), [OC 12-07](#)
Mirab Samiee S. [OC 15-07](#)
Mirabello L. [MTC 03 II-01](#), [SS 09-02](#)
Mirghani H. [HN 08-08](#)
Mishra G. [OC 14-14](#)
Misseri Y. [SSim 02-06](#)
Mita A. [HN 01-03](#)
Mitchell J. [MTC 03 II-01](#)
Mitra A. [OC 11-16](#), [SS 09-05](#)
Mitsunori K. [P12-23](#)
Miyagi E. [WACC I-06](#), [WACC II-12](#), [WACC II-18](#), [WACC II-21](#)

Mobini Kesheh M. [OC 15-07](#)
Moeller E. [OC 13-12](#), [OC 13-13](#)
Moen K. [WACC II-03](#)
Moes-Sosnowska J. [P07-11](#)
Molijn A. [OC 11-09](#)
Moltu P. [OC 01-03](#)
Moncur S. [SS 11-06](#)
Mondal R. [HN 09-06](#)
Money D. [OC 06-09](#)
Monsanto H. [OC 06-11](#)
Moodley J. [OC 01-08](#)
Moore C. [OC 01-05](#)
Morales C. [P12-19](#)
Morato J. [SSim 02-06](#)
Moreau M. [OC 01-07](#), [P12-21](#)
Moreau J.C. [P04-01](#), [P17-03](#)
Moreira E. [OC 13-12](#)
Moreira P.M. [P04-01](#), [P17-03](#)
Morel H.P. [P12-08](#)
Morgan R. [HN 09-02](#)
Morgan K. [WACC II-23](#)
Morimoto A. [WACC II-13](#)
Morin-Papunen L. [SS 09-05](#)
Morrow M. [Ssim 02-07](#)
Morton D. [OC 02-06](#), [OC 12-05](#)
Moscicki A.B. [OC 04-03](#), [SS 10-01](#), [SS 13-01](#)
Moss J. [WACC II-05](#)
Mossong J. [OC 14-08](#), [P10-01](#)
Motlagh A. [OC 15-07](#)
Motoki Y. [WACC I-06](#), [WACC II-18](#), [WACC II-21](#)
Mounier-Jack S. [OC 13-15](#), [WACC II-11](#)
Mourareau C. [HN 08-04](#)
Mouraz M. [P11-02](#)
Moysiadis T. [OC 08-02](#), [OC 10-06](#)
Muck-Wikland E. [HN 03-01](#)
Mueller V. [OC 07-08](#)
Mukhopadhyay A. [HN 04-02](#)
Mulholland E.K. [OC 06-10](#)
Muller T. [WACC II-19](#)
Müller M. [SSim 02-03](#)
Mungdee J. [OC 05-08](#)
Muñiz G. [P15-02](#)
Munk C. [OC 09-01](#), [P07-15](#), [SS 12-02](#)
Munoz N. [OC 13-04](#)

Muñoz F. [OC 13-16](#)
Münstedt K. [GS 03-02](#)
Murillo R. [WACC I-02](#)
Music R. [OC 08-09](#)
Mustafa G. [P12-14A](#), [P12-14B](#), [P12-15B](#)
Mustafa M.E. [P12-14A](#), [P12-14B](#), [P12-15B](#)
Musumeci R. [OC 05-01](#), [P05-03](#)
Mwenitete I. [OC 12-05](#)
Myers J.S. [P11-10](#)
Myers E. [SS 01-01](#)
Myrvang E. [P08-02](#)

N

Naber S. [OC 02-01](#), [OC 02-04](#), [OC 13-06](#), [OC 14-02](#)
Naber S.K. [OC 14-01](#)
Nadeau C. [OC 08-08](#)
Nadji S.A. [OC 15-07](#)
Nagy L. [OC 13-07](#), [OC 13-09](#)
Nakamura A. [P12-20](#)
Nakashima T. [P04-05](#)
Nakashima K. [P04-05](#), [P10-02](#), [P18-02](#)
Nakic D. [P17-09](#)
Nam J.H. [P01-04](#)
Namiki M. [P04-05](#), [P18-02](#)
Narongchai T. [OC 15-03](#)
Näsman A. [HN 03-01](#)
Nassereddine H. [P12-18](#)
Navarro A. [SSim 02-06](#)
Navarro Rodriguez R. [OC 06-11](#)
Nawrocki-Raby B. [HN 08-04](#)
Neagu C.E. [WACC II-14](#)
Neilson A. [CS 01-03](#)
Neis K.J. [OC 09-03](#)
Neophytou P. [OC 01-01](#)
Netterlid E. [WACC II-17](#)
Neumann F. [SS 13-05](#)
Nevermann N. [OC 11-06](#)
Nevéus T. [WACC II-08](#)
Ng B. [OC 04-01](#)
Nguyen C.D. [OC 06-10](#)
Nguyen N. [SS 13-07](#)
Nguyen-Tan P.F. [HN 08-03](#)
Niang M.M. [P04-01](#)

Nicolau B. [HN 08-03](#)
Nicula B. [P17-07](#)
Nicula F. [P17-07](#), [WACC II-03](#)
Niebler M. [SSim 01-03](#)
Nizard M. [HN 07-01](#)
Nkechi O. [P17-05](#), [SS 05-03](#)
Noizat C. [HN 07-03](#)
Nolde N. [P17-04](#)
Nonenoy S. [SS 12-05](#)
Nordin A. [P01-07](#)
Nordqvist Kleppe S. [SS 09-06](#)
Norrild B. [SS 13-02](#)
Nouws S. [OC 05-03](#)
Noy M.A. [P07-12](#)
Nygaard M. [P07-15](#)
Nygård M. [OC 01-03](#), [OC 08-06](#), [OC 14-11](#), [OC 14-12](#), [P07-06](#)
Nyíri M. [OC 11-11](#), [OC 12-13](#)
Nyttray A. [P17-11](#)
Nyttray A.G. [SS 05-03](#)

O

Oberaigner W. [OC 14-15](#)
Obermoser C. [P12-14A](#), [P12-15B](#)
Obermueller T. [OC 11-05](#)
Obón Castel B. [P09-02](#), [P09-03](#)
O'brien M.A. [OC 06-11](#)
Ofstad T. [OC 02-10](#)
Ogilvie G. [OC 06-09](#), [OC 09-06](#)
Oguejiofor K. [HN 09-08](#)
Ohashi Y. [OC 12-17](#)
Olausson P. [OC 14-09](#)
Olivier S. [Ssim 03-04](#)
Olsen A. [WACC II-19](#)
Olson C.G. [OC 04-02](#)
Olszewski W. [P07-11](#)
Oncins R. [OC 08-03](#), [P15-02](#)
O'neill A. [HN 04-03](#)
Onwuka N. [P17-11](#)
Oosterhuis K. [P20-01](#)
Ordi J. [CS 03-03](#), [OC 11-09](#), [OC 12-08](#)
Orescanin V. [P15-05](#)
Oscarsson M. [WACC II-08](#)
Osen W. [SSim 02-05](#)

Östensson E. [OC 02-02](#), [OC 12-07](#), [P12-10](#)
Oštrbenk A. [OC 10-03](#)
O'sullivan B. [HN 02-05](#)
Overbeek L. [OC 02-01](#), [OC 14-02](#)
Overbeek L.I.H. [OC 02-04](#), [OC 14-01](#)
Øvestad I.T.Ø [P17-10](#)
Owens D. [P10-03](#)
Oyola S. [Ssim 02-07](#)

P

Paavonen J. [CS 03-05](#), [MSS 06 A-04](#), [P19-03](#)
Padalko E. [OC 01-04](#)
Paetau A. [P19-03](#)
Pai S. [HN 04-02](#), [HN 04-03](#)
Pai? R. [P17-07](#)
Palacios S. [OC 11-14](#)
Palefsky J. [SS 12-05](#)
Palmer T. [CS 01-01](#), [MTC 03 I-10](#), [OC 01-05](#)
Palmer J. [OC 04-11](#)
Pan J. [CS 01-01](#)
Panicker G. [SS 11-03](#)
Pankakoski M. [OC 14-05](#)
Pankam T. [SS 12-05](#)
Panotopoulou E. [P07-14](#)
Pansa L. [P12-25](#)
Pap K. [OC 11-11](#)
Papagiannakis E. [OC 04-02](#)
Papanikolaou A. [P02-02](#)
Pappa K.I. [OC 11-10](#), [P15-01](#)
Pappa K. [OC 11-13](#), [P15-04](#)
Pär Sparén P. [OC 02-03](#)
Paraskevaidi M. [OC 11-16](#)
Paraskevaidis E. [MTC 03 I-06](#), [OC 04-01](#)
Paredes B. [P02-01](#)
Park J.S. [P12-11](#), [SSim 02-04](#)
Park C. [OC 13-05](#)
Park K.S. [SSim 02-04](#)
Parvu V. [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Pascual-Garcia R. [P07-05](#)
Paskett E. [WACC II-20](#)
Passos M.R.L. [P08-01](#)
Patterson B. [HN 09-02](#), [OC 01-11](#), [OC 08-10](#)
Paulussen T. [WACC II-15](#), [WACC II-16](#)

Pavelyev A. [OC 06-11](#), [OC 13-07](#), [OC 13-09](#)
Pawlita M.P. [OC 01-02](#)
Pawlita M. [GS 03-02](#), [MTC 03 I-07](#), [OC 07-04](#), [P07-09](#)
Paz J.R. [P07-12](#)
Peacock S. [OC 09-06](#)
Pearmain P. [STC 02 I-06](#)
Pedersen H. [OC 10-01](#), [OC 12-01](#), [OC 12-19](#)
Peiró G. [P07-04](#)
Pellegrino A. [OC 06-06](#)
Perez G. [OC 06-08](#)
Perez Amaya G. [OC 13-04](#)
Perez-Mies B. [P12-09](#)
Peris M. [WACC II-23](#)
Persing D. [OC 01-08](#)
Pesic A.P. [OC 01-02](#)
Pesic A. [OC 01-09](#), [OC 12-03](#)
Petchjorm S. [OC 05-08](#)
Peters K. [OC 06-06](#)
Petignat P. [OC 08-07](#), [OC 12-11](#)
Peto J. [OC 09-09](#), [SS 09-01](#)
Petrozza V. [P12-05](#)
Petry K. [OC 06-04](#)
Petry K.U. [GS 03-02](#), [OC 06-05](#), [OC 06-06](#), [OC 13-08](#), [P05-01](#), [SS 12-09](#)
Pfister H. [OC 01-09](#)
Phanupak N. [SS 12-05](#)
Phanupak P. [SS 12-05](#)
Phoolcharoen N. [OC 12-12](#)
Piana A. [OC 05-01](#)
Piao J. [P08-03](#)
Pichi B. [P12-03](#)
Pierce R. [HN 04-02](#)
Pillsbury M. [OC 03-01](#), [OC 06-11](#), [OC 13-05](#), [OC 13-07](#), [OC 13-09](#)
Pils S. [GS 02-02](#)
Piltonen T. [SS 09-05](#)
Pimple S. [OC 14-14](#)
Pinto C. [OC 05-02](#)
Pircher H. [OC 08-11](#)
Pirrotte E. [P10-03](#)
Pitis S. [P02-02](#)
Pittel B. [OC 14-07](#)
Plaza F. [P12-19](#)
Plisko O. [OC 04-05](#)
Ploner A. [OC 05-06](#)
Plummer M. [MTC 01-01](#)

Poethko-Müller C. [P07-09](#)
Poitras N. [OC 08-05](#), [OC 09-08](#)
Polette M. [HN 08-04](#)
Politis G. [P07-14](#)
Poljak M. [HN 08-02](#), [MTC 03 I-07](#), [OC 10-03](#), [OC 15-02](#), [P05-02](#)
Pollock K. [CS 01-01](#), [CS 01-02](#), [CS 01-04](#), [MTC 03 I-10](#), [OC 07-07](#)
Polman N. [SS 08-06](#)
Polman N.J. [OC 04-08](#)
Ponce Lorenzo J. [P07-04](#)
Popadiuk C. [OC 08-08](#)
Poppe W. [P03-02](#)
Porn A.C. [P04-03](#)
Powell N. [P10-03](#)
Pradat P. [P07-02](#)
Praet M. [P14-01](#)
Prato R. [OC 05-07](#)
Préaubert L. [OC 04-10](#)
Preisler S. [OC 12-01](#), [OC 12-02](#)
Preti M. [STC 01 A-06](#)
Pries R. [HN 08-01](#)
Prieske K. [OC 07-08](#)
Prigge E.S. [HN 08-05](#), [HN 09-05](#), [OC 11-05](#), [SSim 02-05](#)
Primic Žakelj M. [OC 14-17](#)
Primic Žakelj M. [P17-04](#)
Profozic Z. [P04-04](#)
Profozic V. [P04-04](#)
Prohctect/improve Study Team* P.S.T. [MTC 03 II-05](#)
Proudlove C. [P12-21](#)
Prusaczyk A. [P07-11](#)
Puggioni C. [P12-05](#)
Pukkala E. [P07-01](#)
Puthanakit T. [OC 06-06](#)

Q

Qendri V. [OC 03-06](#)
Quabius E.S. [HN 08-01](#), [HN 08-06](#)
Queipo Gutierrez F.J. [OC 12-14](#)
Quinlan D. [OC 09-06](#)
Quint W. [CS 03-03](#), [MTC 03 I-07](#), [OC 07-04](#), [OC 11-07](#), [OC 11-09](#), [OC 12-08](#), [OC 12-15](#)
Quiterio M. [P07-17](#), [SS 12-08](#)

R

Rabaan A. [OC 08-01](#)
Rafai E. [OC 06-10](#)
Ragusa A.F. [OC 04-06](#), [OC 15-01](#)
Raine-Bennett T. [MTC 03 II-01](#)
Rajendra S. [OC 07-02](#)
Ramon Cajal J.M. [OC 12-14](#)
Ramón Cajal J.M. [P09-02](#), [P09-03](#)
Ramos S. [WACC II-23](#)
Ramqvist T. [HN 03-01](#)
Ratu T. [OC 06-10](#)
Rautava J. [SS 10-05](#), [SS 12-03](#)
Read C. [P16-01](#)
Rebojl M. [OC 12-02](#)
Rebolj M. [OC 12-01](#), [OC 12-19](#)
Regauer S. [P01-05](#)
Reich O. [P01-05](#)
Reichhuber C. [OC 01-06](#), [OC 01-09](#)
Reiter P. [WACC II-05](#)
Rekosz M. [P07-11](#)
Renschmidt C. [P07-09](#)
Repse Fokter A. [OC 14-17](#)
Resende L. [OC 13-01](#)
Reuschenbach M. [HN 08-05](#), [HN 09-05](#), [OC 11-05](#), [SSim 01-06](#), [SSim 02-05](#)
Reuter C. [SS 06-03](#), [SS 09-01](#)
Revets H. [P12-16](#)
Reyburn R. [OC 06-10](#)
Rezeberga D. [OC 04-05](#)
Rheault P. [OC 06-06](#)
Ribalta J. [P01-09](#), [P04-06](#)
Ribeiro R. [P12-24](#)
Richter K. [WACC II-23](#)
Ridder R. [OC 04-08](#)
Rieder D. [P12-14A](#), [P12-14B](#), [P12-15B](#)
Riethmuller D. [OC 06-05](#)
Rimer B. [WACC II-05](#)
Rincon-Orozco B. [SSim 01-03](#)
Rintala M. [OC 07-03](#)
Ritter M. [OC 13-12](#), [OC 13-13](#)
Riva C. [HN 09-03](#)
Rivera I. [P07-12](#)
Roberson D. [MTC 03 II-01](#)
Roberston C. [CS 01-04](#)
Robertson C. [CS 01-03](#)
Rocha M. [OC 07-09](#), [P11-02](#)

Rochefort J. [HN 07-03](#)
Röcken C. [HN 08-06](#)
Rodbamrung P. [SS 12-05](#)
Rodenburg W. [MSS 01 B-02](#), [OC 14-13](#), [OC 14-16](#)
Rodolakis A. [CS 06-03](#)
Rodriguez A. [CS 03-03](#), [OC 11-09](#), [OC 12-08](#), [SS 09-05](#)
Rodríguez-Cabezas M.E. [P13-01](#)
Rodríguez-Nogales A. [P13-01](#)
Rollo F. [P12-03](#)
Romero I. [P12-12](#)
Romundstad P.R. [OC 14-06](#)
Ronco G. [MSS 03-01](#), [MTC 03 I-08](#), [SS 08-07](#)
Rosado A. [P07-12](#)
Rosado Buzzo A. [OC 06-11](#)
Rosenblad A. [WACC II-08](#)
Rosini S. [P07-16](#), [P12-25](#)
Rösl F. [SSim 01-03](#), [SSim 02-03](#)
Ross M. [P17-11](#), [SS 05-03](#)
Ross I. [WACC II-10](#)
Rounge T. [P08-02](#)
Rousseau M.C. [HN 08-03](#)
Roussel H. [HN 07-01](#)
Roy M. [CS 02-05](#), [CS 04-03](#), [STC 01 A-05](#)
Royackers K. [P06-01](#)
Royannez-Drevard I. [OC 08-07](#)
Rozemeijer K. [OC 02-01](#), [OC 02-04](#), [OC 13-06](#), [OC 14-01](#), [OC 14-02](#)
Rozendaal L. [OC 09-07](#), [OC 11-07](#), [OC 12-15](#)
Ruiz Campo L. [OC 12-14](#), [P09-02](#), [P09-03](#)
Ruiz-Veramendi M. [P12-02](#)
Runnebaum I.B. [OC 11-01](#), [OC 11-12](#)
Rupp F. [OC 01-06](#)
Russell F.M. [OC 06-10](#)
Rygaard C. [OC 12-01](#), [OC 12-02](#), [OC 12-19](#), [P01-06](#), [P17-08](#)
Ryser M.D. [SS 01-02](#)

S

Saah A. [OC 03-01](#), [OC 03-03](#), [OC 06-08](#), [OC 13-03](#), [OC 13-04](#)
Sablone F. [P07-16](#), [P12-25](#)
Saeloo S. [OC 05-08](#)
Sáez A. [P12-12](#)
Safaeian M. [SSim 01-01](#)
Sahiratmadja E. [SS 09-07](#)
Saidu R. [OC 01-08](#)

Sakuragi N. [P12-20](#), [P12-23](#)
Saldanha C. [OC 05-02](#)
Salembier G. [OC 10-04](#)
Saliha H. [OC 10-04](#)
Salmeron J. [P07-17](#), [SS 12-08](#)
Salmerón J. [P02-01](#)
Šaltyte Benth J. [P08-02](#)
Sand F.L. [SS 12-02](#), [SS 13-02](#)
Sandri M.T. [OC 10-01](#), [P12-13](#), [P17-06](#)
Sangrajrang S. [OC 08-04](#)
San-Martin M. [P07-05](#)
Santana I. [OC 07-09](#)
Santegoets S. [HN 07-02](#)
Santos Salas Vailén J. [OC 07-04](#)
Sanusi B. [P11-10](#)
Sargent A. [OC 09-09](#)
Sarkeala T. [OC 14-03](#), [OC 14-05](#)
Sarkola M. [OC 07-03](#)
Sasagawa T. [P18-02](#)
Sasieni P. [MSS 02-01](#), [OC 08-09](#), [OC 09-03](#), [SS 06-05](#)
Sathiya K. [OC 15-03](#)
Sato H. [P12-20](#)
Sattayarungsee P. [OC 05-08](#)
Sauer M. [SSim 01-06](#)
Sauvageau C. [OC 06-09](#), [P11-05](#)
Saville M. [MSS 03-02](#)
Schäfer A.P.A. [GS 03-02](#)
Schaff Z. [OC 11-11](#)
Schausberger C. [P12-14B](#), [P12-15B](#)
Schejter E. [STC 01 A-04](#)
Schellenbacher C. [GS 02-01](#)
Schepe G. [P20-01](#)
Scheungraber C. [OC 11-01](#), [OC 11-12](#)
Schiffman M. [MTC 03 II-01](#), [OC 08-05](#), [OC 09-08](#), [SS 09-02](#)
Schiller D. [OC 11-06](#)
Schilling A. [OC 13-12](#), [OC 13-16](#)
Schim Van Der Loeff M. [WACC II-15](#), [WACC II-16](#)
Schlecht N.F. [HN 08-03](#)
Schmalfeldt B. [OC 07-08](#)
Schmeler K. [P12-07](#)
Schmidt D. [GS 03-02](#), [SSim 01-06](#)
Schmitt F. [OC 14-08](#), [P10-01](#)
Schmitz M. [OC 11-01](#)
Schneider A. [GS 03-02](#), [OC 08-02](#), [OC 10-06](#), [OC 11-06](#)

Schröter J. [P07-09](#)
Schulz T. [SS 12-09](#)
Schurink-Van 't Klooster T.M. [OC 03-04](#)
Schuuring E. [OC 11-02](#), [OC 12-16](#)
Schuyleman A. [MSS 05-05](#)
Schwarz E. [OC 11-12](#)
Schwarz T.F. [OC 03-02](#)
Schwarz T. [OC 06-06](#)
Schweizer J. [HN 09-07](#)
Scott K. [P12-04](#)
Šefčíková A. [P03-01](#)
Segnan N. [MSS 01 A-05](#), [STC 02 I-07](#)
Sehnálková E. [P03-01](#)
Seiwert T. [HN 04-02](#)
Sekine M. [WACC I-06](#), [WACC II-13](#)
Seme K. [OC 10-03](#)
Seo Y.B. [SSim 02-04](#)
Serrano A.M. [P19-02](#)
Serrano I. [CS 01-01](#), [OC 01-07](#)
Serroyen J. [P20-01](#)
Sesia M. [P17-06](#)
Sessa F. [HN 09-03](#)
Sharif Kashani S. [OC 15-07](#)
Sharma P. [OC 07-02](#)
Shastri S. [OC 14-14](#)
Shiao S. [HN 01-03](#)
Shigehara K. [P04-05](#), [P18-02](#)
Shimamura M. [P04-05](#)
Shohei K. [P10-02](#)
Shults K. [OC 08-10](#)
Sichero L. [OC 07-06](#)
Sideri M. [P17-06](#)
Siebers A. [OC 02-01](#), [OC 12-09](#), [OC 14-02](#)
Siebers A.G. [OC 02-04](#), [OC 14-01](#)
Siebert U. [GS 03-02](#), [OC 14-15](#)
Siegler E. [CS 04-04](#)
Silfverschiöld M. [OC 02-02](#)
Silvia M. [OC 06-10](#)
Silva R. [SS 12-08](#)
Silveira F.A. [P08-01](#)
Šimková L. [P03-01](#)
Simmons D. [OC 10-05](#)
Simms K. [SS 03-08](#), [SS 04-02](#)
Singh I. [OC 14-07](#)

Sinha R. [OC 06-10](#)
Siriporn S. [OC 15-03](#)
Sivaram S. [SS 01-03](#)
Sivars L. [HN 03-01](#)
Skar R. [SS 13-04](#)
Skenderi A. [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Skorstengaard M. [P01-06](#)
Skov Jensen J. [P17-01](#)
Smart L. [OC 01-05](#)
Smetansky V. [P03-01](#)
Smieja M. [HN 09-07](#)
Smith M. [SS 03-08](#), [SS 04-02](#)
Smith S.B. [WACC II-23](#)
Smith L. [OC 09-06](#)
Smith J. [P11-10](#)
Smith J.H. [OC 10-02](#)
Smith J.S. [WACC II-23](#)
Smith-Mccune K. [OC 04-03](#)
Smola S. [ES-02](#)
Sneige N. [P12-07](#)
Snijders P. [MTC 03 I-07](#), [OC 11-07](#), [OC 11-17](#), [OC 12-09](#), [OC 12-10](#), [OC 12-15](#), [SS 03-01](#), [SS 08-06](#)
Snijders P.J.F. [MTC 03 II-03](#), [OC 02-08](#), [OC 04-08](#), [OC 09-07](#), [OC 11-03](#)
Sobel G. [OC 11-11](#)
Soboljeva , O. Petronchak . R. Huley, I. Palyha V. [P11-07](#)
Sofia A. [OC 07-09](#)
Sokari T. [WACC II-20](#)
Soldano F. [SSim 01-06](#)
Somers E. [OC 10-04](#)
Sonder G. [WACC II-15](#), [WACC II-16](#)
Song Y. [OC 11-18](#)
Sorbe B. [P01-03](#)
Sørbye S.W. [OC 02-10](#)
Sornsamdang G. [OC 12-12](#)
Sotgiu G. [OC 05-01](#)
Soubeyrand B. [OC 06-04](#), [OC 06-05](#)
Soulières D. [HN 08-03](#)
Sousa M. [P12-01](#), [P12-24](#)
Soutschek E. [OC 01-06](#), [OC 01-09](#), [OC 08-02](#), [OC 08-11](#), [OC 10-06](#)
Souza L.C. [OC 06-07](#)
Spaczynski M. [OC 03-02](#)
Sparen P. [OC 02-05](#), [STC 02 I-05](#)
Sparén P. [OC 02-02](#), [OC 05-06](#), [P07-08](#), [SS 09-06](#), [WACC II-17](#)
Speck N. [P04-06](#)
Spolti N. [P17-06](#)

Spriano G. [P12-03](#)
Spruijt J.W.M. [OC 04-08](#)
Spruijt J.W. [OC 11-03](#)
Spruijt J. [OC 11-07](#), [OC 12-15](#)
Sricharunrat T. [OC 05-08](#)
Sroczynski G. [OC 14-15](#)
Staebler A. [OC 09-03](#)
Staerkel G. [P12-07](#)
Stanczuk G.A. [OC 12-04](#)
Stanczuk G. [OC 12-05](#)
Stanley M. [OC 06-04](#), [OC 06-05](#)
Steben M. [SS 02-06](#), [SS 04-06](#), [STC 01 A-03](#)
Steenbergen R. [OC 11-02](#), [OC 11-17](#), [OC 12-10](#), [OC 12-15](#)
Steenbergen R.D.M. [OC 02-08](#), [OC 04-08](#), [OC 11-03](#)
Steinberg B. [HN 05-01](#)
Stenhammar C. [WACC II-06](#), [WACC II-08](#)
Šterbenc A. [OC 10-03](#), [OC 15-02](#)
Stern P. [ES-01](#), [HN 09-08](#)
Sterzing F. [OC 11-05](#)
Stewart J. [SS 11-06](#)
Stieber D. [P10-01](#)
Stock S. [OC 11-16](#)
Stokley S. [MSS 06 A-01](#)
Stoler M. [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Storhaug S. [WACC II-10](#)
Strehlke S. [OC 13-08](#), [P05-01](#), [SS 12-09](#)
Strojan P. [HN 08-02](#)
Struyf F. [OC 03-02](#), [OC 03-05](#), [OC 06-06](#)
Stubenrauch F. [GS 01-07](#)
Sturm L. [WACC II-07](#)
Suarez A. [P12-09](#)
Subasinghe A. [SS 13-06](#), [SS 13-07](#)
Sudenga S. [P07-17](#), [SS 12-08](#)
Sudenga S.L. [OC 07-06](#)
Sukegawa A. [WACC I-06](#), [WACC II-21](#)
Sundström K. [SS 09-06](#)
Sung Y.C. [SSim 02-04](#)
Surcel H.M. [P07-01](#)
Suryakiran P. [OC 06-06](#)
Suryakiran P.V. [OC 03-02](#)
Susanto H. [SS 09-07](#)
Šustek A. [P03-01](#)
Suteu O. [P17-07](#), [WACC II-03](#)
Suzuki Y. [WACC I-06](#), [WACC II-18](#), [WACC II-21](#)

Suzuki M. [HN 08-07](#)
Svahn M.F. [SS 12-02](#)
Svanholm H. [P17-01](#), [P17-08](#)
Svanholm-Barrie C. [OC 01-08](#)
Syrjänen S. [HN 01-01](#), [OC 07-03](#), [SS 12-03](#)
Syrjänen K. [CS 04-02](#), [OC 07-01](#), [OC 07-03](#), [SS 12-03](#)
Székely I. [OC 11-11](#), [OC 12-13](#)
Szentirmay Z. [OC 13-07](#), [OC 13-09](#)

T

Tabrizi S.N. [OC 03-08](#), [OC 06-10](#), [SS 12-07](#)
Tabrizi S. [P05-02](#)
Tachezy R. [OC 01-01](#)
Tae-Joong K. [P01-01](#), [P01-08](#)
Taepisitpong C. [OC 05-08](#), [OC 12-12](#)
Takac I. [OC 14-17](#)
Takács T. [OC 11-11](#), [OC 12-13](#)
Takata T. [WACC II-13](#)
Takayuki S. [P12-23](#)
Tanaka K. [HN 07-03](#)
Tanaka Y. [WACC II-13](#)
Tang E. [SS 12-05](#)
Tang R.B. [OC 06-06](#)
Tantitamit T. [OC 02-09](#), [OC 08-04](#)
Tanzi E. [OC 05-01](#)
Tapp J. [P10-01](#)
Tartour E. [HN 07-01](#), [HN 07-03](#)
Taxa L. [OC 04-09](#)
Tay S.K. [WACC II-22](#)
Teather C. [OC 10-02](#)
Teeratakulpisarn N. [SS 12-05](#)
Teerayathanakul N. [OC 05-08](#)
Téllez-Sosa J. [OC 11-15](#)
Tenet V. [SS 09-02](#)
Ter Haar R. [OC 02-06](#), [OC 12-05](#)
Ter Harmsel B. [CS 03-03](#)
Ter Harmsel A. [OC 11-07](#), [OC 12-15](#)
Terebessy A. [WACC II-04](#)
Tergas A. [OC 01-08](#)
Termrungruenglert W. [OC 02-09](#), [OC 08-04](#), [OC 13-17](#)
Tertipis N. [HN 03-01](#)
Tetteh S. [OC 12-03](#)
Thamm M. [P07-09](#)

Thamsborg L.H. [P17-08](#)
Thaniya S. [OC 15-03](#)
Theodoridis T. [P11-06](#)
Thibaut I. [P12-06](#)
Thiessen M. [OC 01-06](#), [OC 01-09](#)
Thomas F. [OC 03-02](#), [OC 06-06](#)
Thomas S. [MSS 05-05](#)
Thomas T. [HN 06-02](#)
Thomsen L. [OC 12-01](#)
Thomsen L.T. [OC 09-01](#), [P07-15](#)
Thorsen L. [WACC II-10](#)
Ticic V. [P07-10](#)
Tidy J. [CS 02-04](#), [OC 04-11](#)
Tikoduadua L. [OC 06-10](#)
Tincu A. [WACC II-03](#)
Tinhofer-Keilholz I. [HN 09-05](#)
Tjalma W.A. [P06-01](#)
Tjalma W. [OC 10-04](#), [OC 13-10](#), [SS 09-04](#)
Todescu A. [P17-07](#)
Toh Z.Q. [OC 06-10](#)
Tokugawa D. [OC 09-08](#)
Toma A. [OC 06-06](#)
Tommasino M. [HN 09-04](#), [MTC 03 I-07](#), [MTC 03 II-03](#)
Tommola P. [P19-03](#), [STC 01 B-01](#)
Tomsic S. [OC 04-12](#)
Tonetti A. [OC 04-06](#), [OC 15-01](#)
Törnberg S. [OC 14-09](#), [SS 08-05](#)
Torne A. [CS 03-03](#), [OC 11-09](#), [OC 12-08](#)
Torres B.N. [SS 12-08](#)
Torres-Ibarra L. [P02-01](#)
Torres-Poveda K.J. [OC 11-15](#)
Toshiyuki S. [P10-02](#)
Tota J. [OC 03-05](#)
Totain E. [SSim 02-06](#)
Totchiyev G. [P18-01](#)
Tran T. [HN 07-01](#)
Tranberg M. [P17-01](#)
Trauner A. [P12-14A](#), [P12-14B](#), [P12-15B](#)
Tricca A. [P17-06](#)
Trillsch F. [OC 07-08](#)
Trobe A. [OC 14-12](#)
Tropé A. [OC 08-06](#), [OC 14-11](#), [WACC II-10](#)
Trottier H. [CS 04-01](#), [MTC 03 II-06](#)
Truong Y. [WACC II-05](#)

Tshomo U. [MTC 03 II-03](#)
Tsimplaki E. [P07-14](#)
Tsolakidis D. [P02-02](#)
Tsuyoshi S. [P12-23](#)
Tuivaga E. [OC 06-10](#)
Tummers B. [SSim 01-05](#)
Twitty C. [HN 04-02](#)
Tydén T. [WACC II-08](#)
Tzeng J.Y. [P04-07](#)

U

Ueda Y. [WACC I-06](#), [WACC II-13](#)
Uhan Kastelic S. [P17-04](#)
Uhart M. [P01-07](#), [P11-09](#)
Uhnou I. [WACC II-17](#)
Uijterwaal M.H. [OC 04-08](#), [OC 11-03](#)
Umulisa M.C. [MTC 03 II-03](#)
Unkila-Kallio L. [P19-03](#)
Urban K. [SSim 02-05](#)

V

Vaknin Z. [CS 04-04](#)
Valentine K. [OC 14-04](#)
Valladares W. [P15-03](#)
Vallés V. [P15-02](#)
Van Agt H.M.E. [OC 02-04](#), [OC 14-01](#)
Van Agt H. [OC 02-01](#), [OC 14-02](#)
Van Baal M. [OC 11-07](#), [OC 12-15](#)
Van Baal M.W. [OC 04-08](#), [OC 11-03](#)
Van Ballegooijen M. [OC 02-01](#), [OC 02-04](#), [OC 13-06](#), [OC 14-01](#), [OC 14-02](#)
Van Beek J. [OC 15-04](#)
Van Criekinghe W. [OC 11-02](#)
Van Damme P. [MSS 05-05](#), [OC 06-04](#), [OC 06-05](#), [OC 13-12](#), [P12-16](#), [P12-17](#), [P12-21](#), [P12-22](#), [SSim 03-04](#)
Van De Sandt M. [CS 03-03](#), [OC 11-09](#)
Van Den Brule A.J. [OC 15-04](#)
Van Der Burg S.H. [SSim 01-02](#)
Van Der Burg S. [HN 07-02](#), [SSim 01-05](#)
Van Der Linden J.C. [OC 15-04](#)
Van Der Maas N.A. [OC 03-04](#)
Van Der Veen N. [MSS 01 B-02](#), [OC 14-13](#), [OC 14-16](#)
Van Der Velden L.A. [HN 07-02](#)

Van Der Zee A.G.J. [OC 12-16](#)
Van Der Zee A. [OC 11-02](#)
Van Diermen Hidle B. [OC 01-03](#)
Van Dijken D. [OC 11-07](#), [OC 12-15](#)
Van Dijken D.K.E. [OC 04-08](#), [OC 11-03](#)
Van Gucht S. [MTC 03 I-07](#)
Van Ham V. [HN 07-02](#)
Van Keer S. [P12-16](#), [P12-17](#), [P12-21](#), [P12-22](#)
Van Kemenade F. [CS 03-03](#), [OC 11-07](#), [OC 11-09](#), [OC 12-09](#), [OC 12-10](#), [OC 12-15](#), [OC 14-02](#)
Van Kemenade F.J. [OC 02-04](#), [OC 04-08](#), [OC 09-07](#), [OC 11-03](#), [OC 14-01](#)
Van Laere E. [P16-02](#)
Van Leeuwen R. [OC 11-02](#)
Van Leeuwen R.W. [OC 12-16](#)
Van Loon A. [OC 14-16](#)
Van Niekirk D. [OC 09-06](#)
Van Ostade X. [P12-22](#), [SS 09-04](#)
Van Raemdonck G. [SS 09-04](#)
Van Roost A. [P06-01](#), [P12-17](#)
Van Rosmalen J. [OC 13-06](#)
Van Zummeren M. [OC 02-08](#), [OC 09-07](#), [OC 11-03](#), [SS 03-01](#)
Vanden Broeck D. [MTC 03 I-07](#), [OC 01-04](#), [OC 05-03](#), [OC 14-04](#), [P12-19](#), [P14-01](#)
Vandeweyer K. [P06-01](#)
Vankerckhoven V. [OC 12-08](#), [P12-16](#), [P12-17](#), [P12-21](#)
Vänskä S. [SS 08-04](#)
Vasiljevic N. [SS 06-03](#)
Vassilakos P. [OC 08-07](#), [OC 12-11](#)
Vatopoulou A.N.A.S.T.A.S.I.A. [P11-06](#)
Vatopoulou A. [P02-02](#)
Veldhuijzen N. [MSS 03-04](#), [SS 08-06](#)
Velicer C. [OC 13-04](#)
Vellinga J. [P20-01](#)
Velten M. [P07-02](#)
Verdonck K. [P14-01](#)
Verdoodt F. [MSS 02-06](#), [SS 13-02](#)
Verheijen R.H.M. [OC 04-08](#), [OC 11-03](#)
Verheijen R. [OC 11-07](#), [OC 12-15](#)
Verhoef V. [OC 12-09](#), [OC 12-10](#)
Verschuere H. [P03-02](#)
Verstraeten T. [OC 06-04](#)
Vetter A.S. [OC 01-06](#), [OC 01-09](#)
Veza T. [P13-01](#)
V́ctor V. [OC 08-03](#)
Vieira-Baptista P. [OC 05-02](#)
Vielh P. [HN 08-08](#), [OC 14-08](#)

Vielot N.A. [WACC II-23](#)
Villa L.L. [OC 07-06](#), [OC 14-10](#)
Villa L. [SS 12-08](#)
Vinokurova S. [HN 09-05](#), [OC 11-04](#)
Vinson C. [SS 01-03](#)
Vintermyr O.K. [OC 01-03](#), [SS 13-04](#)
Vinzon S. [SSim 02-03](#)
Virtanen A. [OC 14-03](#)
Viviano M. [OC 08-07](#)
Vladareanu R. [WACC II-14](#)
Vlahou A. [OC 11-10](#), [P15-01](#), [P15-04](#)
Volodko N. [P11-07](#)
Von Knebel Doeberitz M. [GS 01-04](#), [HN 08-05](#), [HN 09-05](#), [OC 11-05](#), [SSim 01-06](#), [SSim 02-05](#)
Von Knebel-Doeberitz M. [OC 11-04](#)
Von Laer D. [OC 14-15](#)
Von Weyhern C.H. [OC 09-03](#)
Voron T. [HN 07-01](#)
Vorsters A. [MTC 03 II-03](#), [P12-16](#), [P12-17](#), [P12-21](#), [P12-22](#)
Vranes J. [P07-10](#)
Vytrisalová P. [P03-01](#)

W

Wagner S. [MTC 03 II-01](#)
Wain G.V. [OC 07-04](#)
Wakeham K. [OC 07-07](#)
Waldstrøm M. [P17-08](#)
Walker G. [OC 02-06](#)
Walker H. [OC 02-06](#)
Walker F. [P12-18](#)
Wang B. [OC 07-02](#)
Wang J. [OC 02-05](#), [P07-08](#), [WACC II-17](#)
Wang R. [OC 11-02](#)
Wang L. [OC 11-18](#), [P07-13](#)
Waraphorn K. [OC 15-03](#)
Wark J. [SS 13-06](#), [SS 13-07](#), [WACC II-18](#)
Warman R. [SS 06-03](#)
Wasanai K. [OC 15-03](#)
Washington C. [WACC II-20](#)
Waterboer T. [HN 02-04](#), [HN 03-02](#), [P07-09](#), [SS 11-05](#)
Watson-Jones D. [OC 13-15](#), [WACC II-11](#)
Wax A. [OC 04-03](#)
Weiderpass E. [P17-07](#), [WACC II-03](#)
Weik M. [P12-14A](#)

Weinstein G. [Ssim 02-07](#)
Weiß V. [OC 13-08](#), [P05-01](#)
Welters M. [HN 07-02](#)
Wennerberg J. [OC 02-02](#)
Wennington H. [CS 01-01](#)
Wentzensen N. [CS 03-01](#), [CS 05-01](#), [GS 03-05](#), [MSS 02-02](#), [MSS 02-06](#), [MSS 03-03](#), [MTC 02-02](#),
[MTC 03 II-01](#), [OC 08-05](#), [OC 09-08](#), [SS 06-04](#), [SS 09-02](#), [SSim 01-01](#), [SSim 01-06](#)
West C. [HN 09-08](#)
Westerling R. [WACC II-08](#)
Weyers S. [OC 14-04](#), [OC 14-08](#)
Whaley K. [WACC II-23](#)
White M. [P17-05](#), [P17-11](#), [SS 05-03](#)
Wichmann O. [P07-09](#)
Widschwendter A. [OC 14-15](#)
Wiebren T. [P12-22](#)
Wiese-Posselt M. [P07-09](#)
Wigan R. [SS 12-07](#)
Wilhelm T. [SS 09-01](#)
Wilkin T. [SS 06-06](#)
William F. [OC 12-04](#)
Williams A. [SS 11-06](#)
Willows K. [OC 13-02](#)
Wirth L. [HN 04-03](#)
Wisman G.B.A. [OC 12-16](#)
Wisman B. [OC 11-02](#)
Wisniewski P. [P07-11](#)
Witkin S.S. [OC 06-07](#)
Witte B.I. [OC 04-08](#), [OC 11-03](#)
Woelber L. [OC 07-08](#)
Wolfson M. [OC 08-08](#)
Woo J.W. [SSim 02-04](#)
Wormenor C.M. [OC 12-18](#)
Wormenor C. [OC 12-03](#)
Woronoff A.S. [SS 13-05](#)
Wright T. [OC 01-08](#), [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Wu R. [HN 05-01](#)
Wu J. [OC 07-02](#)
Wunderlich K. [P20-01](#)
Wunsch K. [OC 11-01](#)
Wysocki J. [OC 03-02](#)

X

Khaja A. [OC 11-08](#)

Xiao Y. [MTC 03 II-01](#)
Xiao-Jun M. [HN 08-08](#)
Xiong Y. [OC 11-18](#)
Xu L. [CS 03-04](#), [MSS 02-06](#)
Xue X. [OC 11-18](#)

Y

Yaegashi H. [P04-05](#), [P18-02](#)
Yagi A. [WACC II-13](#)
Yamaguchi M. [WACC II-13](#)
Yan J. [Ssim 02-07](#)
Yang Q. [MTC 03 II-01](#), [SS 09-02](#)
Yang Z. [Ssim 02-07](#)
Yang W.K. [P01-02](#), [P04-02](#)
Yanson K. [OC 09-02](#), [OC 09-05](#), [SS 12-04](#)
Yasuhide K. [P10-02](#)
Yeager M. [SS 09-02](#)
Yoo-Young L. [P01-01](#), [P01-08](#)
Yoshida E. [HN 01-03](#)
Yoshitomo K. [P10-02](#)
Young E. [HN 09-07](#)
Yu K. [SS 09-02](#)

Z

Zahn R. [P20-01](#)
Zakarneh A. [HN 09-05](#)
Zambrano H. [P12-19](#)
Zappacosta R. [P07-16](#), [P12-25](#)
Zarochentseva N. [P07-03](#), [P11-08](#)
Zeiser I. [OC 11-08](#)
Zekan J. [P17-09](#)
Zhang X. [P17-05](#), [SS 05-03](#), [SS 09-02](#)
Zhang L. [OC 11-18](#), [SSim 01-04](#)
Zhang D. [P11-03](#)
Zhang C. [OC 11-18](#)
Zhang Y. [P08-03](#)
Zhao K.N. [SSim 01-04](#)
Zhao K. [OC 11-18](#)
Zhou H. [P12-07](#)
Zhou F. [SSim 01-04](#)
Zhu B. [MTC 03 II-01](#)
Zhukova O. [P07-07](#)

Zidar N. [HN 08-02](#)

Zimet G. [WACC II-07](#), [WACC II-15](#), [WACC II-16](#), [WACC II-23](#)

Zimmerman M. [SS 12-06](#)

Zingone G. [SSim 02-06](#)

Ž

Žodžika J. [OC 04-05](#)

Z

Zoidakis J. [OC 11-10](#), [P15-01](#), [P15-04](#)

Zorec T.M. [HN 08-02](#)

Zumsteg Z. [HN 01-03](#)