EUROGIN 2017

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Background / Objectives

Different instruments, which are frequently formal documents, are available that stakeholders involved in the population screening use to specify and regulate requirements, tasks, and responsibilities.

Methods

The Ministry of Public Health, Welfare and Sport, the Centre for Population Screening, the screening organisations and the professionals play a role in the guidelines used.

Results

The Ministry of Public Health, Welfare and Sport issues a Dutch Population Screening Act permit. The Dutch Population Screening Act permit has requirements that are needed to protect the participating people from any risks of the population screening. These requirements bear upon the test method, the target group, the screening interval, as well as measures needed to guarantee the quality of the population screening.

The Centre for Population Screening specifies the national quality requirements, indicator set and the frameworks (for example, the information framework) of the population screening. The development of the national requirements and the indicator set is a result of close coordination among the relevant chain stakeholders. The quality requirements are described in a policy and requirements framework (available in September on http://www.rivm.nl/Onderwerpen/B/Bevolkingsonderzoek_baarmoederhalskanker_voor_professionals/Documenten_en_downloads).

The screening organisations have contracts with the screening laboratories. General conditions are in effect with the GPs. These contracts and general conditions comprise the specified agreements regarding the national quality requirements and applicable guidelines. The tasks and responsibilities are also described. This also applies to the desired quality assurance, expertise, data specification, and data exchange. The screening organisations can use the protocol manuals and procedures to provide practical instructions on the implementation aspects in accordance with the national quality requirements.
The Dutch College of General Practitioners (NHG), the Dutch Pathology Association (NVVP), and the Dutch Society for Medical Microbiology (NVMM) develop and maintain guidelines in which the professional, standard and accountable care for their target group is outlined. Components of these guidelines are applicable to the population screening. The Dutch College of General Practitioners (NHG) has developed a practice guide for implementation aspects relevant to the population screening.

Conclusion

The screening program has sufficient methods and guidelines to ensure uniform quality.
CERVICAL CANCER SCREENING GUIDELINES - THE TIMES THEY ARE A' CHANGING: ITALY

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Background / Objectives

In Italy, the HTA report on HPV screening defined the possible new recommendations for public health programs in 2012 (1) and the Italian Ministry of Health received these recommendations in 2013.

The Italian National Prevention Plan 2014-2018 established among the aims for the Regional Health Systems the complete transformation of the cervical cancer screening from Pap test to HPV-based by the end of 2018 (2).

The main points of the new guidelines and the strategies for implementations adopted in Italy will be presented.

Methods

After the inclusion of the HPV as primary screening test for women over 30/35 with 5-year interval and cytologic triage in the Italian recommendations, several tools for implementations have been developed by the National Screening Monitoring Centre (ONS) and the Italian cervical cancer screening scientific society (GISCi).

The ONS conducted a survey on behalf of the Ministry of Health to assess the level of HPV screening implementation in the 20 Italian Regions.

Results

In 2015 16% of the Italian target population have been invited for a HPV test.

Implementation of HPV-based programs differs among regions for starting age (30 or 35) and for the transition strategies.

The level of implementation also differs with regions that already completely converted their screening program and other regions that did not yet completed the administrative steps necessary for identification of centralised HPV test laboratories, for the call for tender to purchase test.

Conclusion

In Italy public screening programs are shifting from Pap test to HPV test-based screening. The regional health systems are affording this change with different strategies and at different speeds. Despite a clear definition of the national goals by
the Ministry of Health, not all the regions probably will reach the target of completing the transition by the end of 2018.

References


CS 02-06
GUIDELINE DEVELOPMENT AND IMPLEMENTATION OF HPV-BASED SCREENING IN SWEDEN

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Background / Objectives

Screening started in the late 1960s in Sweden and was nation-wide by the mid 1970s. The organized, population-based cervical screening program follows national recommendations but is implemented regionally. In 2015, the National Board of Health and Welfare issued new recommendations for cervical cancer that include 3-yearly HPV-based screening for women ages 30-49, 7-yearly HPV-based screening for women 50-64, and 3-yearly cytology-based screening for women 23-29. Guidelines on how to implement the new program were adopted in January 2017 and at the time of submission, primary HPV-based screening for women over the age of 30 is implemented in 3 major regions of Sweden. Further details on the new program, as well as results from the region Stockholm-Gotland where pilot HPV-based screening was started already in 2012 will be presented.

Methods

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Results

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Conclusion

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Background / Objectives

At present in England, cervical screening is recommended every three years for women 25-49 years of age and every five years for women 50-64 years of age. The recommended screening test is liquid-based cytology. Human Papillomavirus (HPV) testing is used for triage of low grade cellular abnormalities and as test of cure after treatment of cervical intraepithelial neoplasia. For now, screening recommendations for vaccinated birth cohorts remain the same as for unvaccinated birth cohorts.

HPV testing is going to replace cytology in primary screening in spring 2019, with reflex cytology being used for triage. An HPV primary screening implementation group consisting of various stakeholders and experts has been established by Public Health England (PHE) to develop plans to roll out this service by the agreed deadline. At present, 59 laboratories provide cytology services to the programme; with HPV testing, it has been decided that the number of screening laboratories will be substantially lower although the decision on the exact configuration is still pending upon an on-going options appraisal. Much attention is given to sample taker training, and guidance is being provided to laboratories in terms of choosing HPV assays.

To support the decision-making, six sites started a pilot implementation of HPV-based primary screening in mid-2013, which is running in parallel with the routine cytology-based screening. These data will help establish the demand for e.g. colposcopy services in a routine setting, and inform the decisions on the screening interval and the clinical follow-up after a positive HPV test result. Furthermore, this pilot will establish the economic cost of the switch from cytology to HPV testing, as well as provide information on its psychological consequences for the women.

The screening programme is at present facing a number of challenges. One is a call-recall system which was established in the late 1980’s. It is currently undergoing a thorough overhaul so that it will be robust enough to support the new HPV-based programme once rolled out. Another challenge is a falling coverage that has been observed in all age groups, with one in every five local authorities reaching less than 70% of the targeted women. This can hopefully be addressed through HPV-based self-sampling, which is one of the current research priorities.

Methods

Results
Conclusion

References

PHE’s screening blog is regularly updated with news regarding HPV-based primary screening: https://phescreening.blog.gov.uk/.
CS 02-08
Cervical cancer screening guidelines - the times they are a' changing

P. Hillemanns

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Background / Objectives

Since 1971, opportunistic screening for cervical cancer has been established in Germany. Women above the age of 20 are offered annual Pap smears (no upper age limit). In 2008, the German Federal Ministry of Health and other organizations launched the “National Cancer Plan”, which is the basis for the Law on Cancer Screening and Registration (KFRG). The KFRG enacted in 2013 demanded the development of an S3 clinical guideline to collect all available evidence on cervical cancer screening in order to define new algorithms for screening and management of cervical dysplasia.

Methods

In 2016, scientific societies of the German S3-guideline group “Cervical Cancer Prevention” published evidence-based statements and recommendations (GRADE approach) with financial support from the German Cancer Aid and under scientific guidance of the German Guideline Program in Oncology (GGPO) and the Association of the Scientific Medical Societies in Germany (AWMF).

Results

Systematic guideline review and its included meta-analyses conducted by two independent institutes (M. Arbyn, WIV-ISP, Belgium; J. Kleijnen, KSR, England) by KSR showed a better protection from cervical cancer and CIN 3+ with HPV screening than with cytological screening. The guideline group preferentially recommends an organized HPV-based screening every 3 - 5 years for all women above 30 years. Women below 30-35 should be screened with cytology. In case of non-participation in the organized screening program, HPV self-sampling should be offered. After a positive HPV screening test, cytology triage is primarily recommended. In case of a positive cytological screening test, HPV testing is the primary recommended triage method. The S3 guideline was published in 2016 at http://awmf.org after a four week online consultation period along with several quality indicators for cervical cancer screening and management. A special guideline version for the public is provided additionally.

Conclusion

The Federal Joint Committee (G-BA) as the highest decision-making body of the joint self-government of physicians, dentists, hospitals and health insurance funds in Germany, issues directives for the benefit catalogue of the statutory health insurance funds (GKV) for more than 70 million insured persons and thus specifies which
services in medical care are reimbursed by the GKV. In 9/2016, the G-BA published cornerstones for future cervical cancer screening in Germany with an organized screening program for women at 35 years of age and older with HPV-Pap co-testing at 3 yearly intervals and yearly Pap testing between 20 – 34 years (2).

References

1. http://leitlinienprogramm-onkologie.de/Zervixkarzinom-Praevention.89.0.html

2. https://www.g-ba.de/institution/presse/pressemitteilungen/641/
Cervical Cancer Screening Guidelines: The view from Canada

E. Franco

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Background / Objectives

N/A

Methods

With its long history of successful organized cervical cancer screening, Canada has been also among the pioneer countries conducting large, publicly funded randomized controlled trials of HPV testing in cervical cancer screening. However, progress in adopting molecular-based technologies in screening has been slow. HPV testing has gained acceptance in Canada only as a strategy for the triage of women with ASCUS smears. The most recent Canadian Task Force on Preventive Health Care (CTFPHC) evidence review (2013) was unfortunately silent on HPV testing and based all its recommendations on Pap cytology. The Pan-Canadian Cervical Cancer Screening Network sponsored a consensus conference in 2014 that concluded that HPV testing should be the anchor technology in cervical cancer screening among vaccinated and unvaccinated populations. Health policymaking in Canada is at the provincial level, however, national or federal recommendations are not binding on the provinces. Although published a year earlier (2012) than the CTFPHC’s guideline, the most progressive and well-informed evidence review was that of the Ontario Cervical Screening Guideline Working Group. It recommended standalone primary HPV testing (with a validated assay for oncogenic types) every five years for women aged 30 to 65 years, with Pap cytology triage of women with a positive HPV test result. In light of the logistical complexities and costs associated with implementing this paradigm change, the Ontario Ministry of Health was able to appropriate the budget and rollout the changes only this year (2017). Quebec completed its evidence review in June 2017, and concluded that a case could be made for a move to primary HPV screening but policy decisions can only be made after a detailed cost-effectiveness analysis via mathematical modelling. This analysis is ongoing. Other provinces are gradually considering changes via pilot projects or evidence reviews. Completion of the British Columbia FOCAL trial is expected to provide strong evidence to assist that province and others to move forward with implementation.

Results

N/A

Conclusion

N/A
References

N/A
Cervical Cancer Screening Guidelines in the US - Current Status and Future Directions

N. Wentzensen

National Institutes of Health (United States of America)

Background / Objectives

Currently, three different primary screening strategies are approved in the US, cytology, HPV-cytology co-testing, and HPV alone.

Methods

These approaches have different intervals and use different triage strategies. In addition, there are management guidelines focusing on screen-positive women and recently, colposcopy recommendations were developed for the US.

Results

Currently, several organizations are preparing updates of screening and management guidelines to adapt to developments in the areas of screening and triage and to address the entering of the first cohorts of vaccinated women into screening programs.

Conclusion

This presentation will review the various current screening, triage and management strategies and will provide an outlook towards the next iteration of guidelines developed by the American Society of Colposcopy and Cervical Pathology which will attempt to simplify screening and management procedures for providers through a risk-based approach and supported by a mobile application.
Turkey: Story of Screening Legend; HPV DNA Results of 2 Million ladies

M. Gultekin

MD (Turkey)

Background / Objectives

Turkey has implemented HPV DNA Testing as a primary screening tool by 2013. This abstract aims to update the current results of screening with initial colposcopy and histology results.

Methods

Women age 30 years and older are invited for cervical cancer screening. All ladies are sampled for via HPV DNA and conventional cytology. Target population totally for 5 years was 15 million. All samples were collected in a central Mega HPV lab in Ankara. For HPV positive cases, additional HPV Genotyping plus cytological evaluations are done. HPV DNA is analysed by HC2, genotyings were done using Genomica kits.

Results

Among evaluated 2 million ladies, HPV positivity rate was 3.8%. The most common type for HPV was 16, followed by 51, 31. Among these cases, most common histological abnormalities were LSIL followed by ASC-US and HSIL. Most common type of colposcopic procedures were punch biopsy. Among screened group, highest peak age of HPV positivity and cytological abnormalities was seen between 35-30. Among all colposcopic biopsies, a great majority of the cases detected were CIN3 (15%), followed by CIN2 and CIN1. Invasive cervical cancer incidence was more than 30/100,000 among screened population compared to 4/100,000 in general Turkish population.

Conclusion

HPV DNA Tests are feasible to apply to general population. Turkey is the first to implement this nationally in a large population for over 15 million ladies. This presentation gives the preliminary results of a nation wide screening.
Background / Objectives

In the Netherlands, a series of self-sampling studies have been performed among screening non-attendees (PROHTECT-1, -2, -3, and -3b), which collectively have shown that offering self-sampling can improve the cervical cancer screening program. Currently, a study among regular screening responders is ongoing (IMPROVE). In this 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 is performed. 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.

Methods

Results

Conclusion

References

*) 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 Babović, JW Spruyt, F Voorhorst, JA Beliën, W Quint; Departments of Pathology and Epidemiology/Biostatistics, VUmc, Amsterdam, Netherlands; Departments of Obstetrics/Gynaecology and Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; Department of Pathology, Erasmus MC University Medical Centre Rotterdam, Netherlands; DDL, Rijkswijk, Netherlands; PALGA, Houten, Netherlands; Screening Organisations Midden-West, Zuid-West and Oost, Netherlands; RIVM, Netherlands.
SELF-SAMPLES AND URINE SAMPLES, SELECTIVE POPULATION VERSUS OPPORTUNITIES FOR ALL

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Background / Objectives

HPV self-sampling and urine HPV testing have the potential to increase cervical cancer screening completion among under- and never-screened women. Cytology and high-risk (hr) HPV testing currently rely on cervical specimens collected by medical personnel during a pelvic examination. Self-sampling of specimens could provide a simple and scalable alternative to physician collection, through non-invasive screening methods that may be more acceptable to women. Screening by self-samples or urine samples could also reduce the need and expense for trained medical personnel to complete primary screening via pelvic exam, with referral of only hrHPV self-test positive women to confirmation and/or treatment.

Self-collected specimens and physician-collected specimens have similar HPV detection results overall. However, a systematic review found that the pooled sensitivity of HPV in self-collected samples was somewhat lower than in physician-collected samples (0·88; 95% CI: 0·85-0·91) for the detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+), with a slightly lower specificity of self-collection (0·96; 0·95-0·97) vs physician HPV testing. Similar sensitivity for self-collection was observed in studies utilizing a PCR-based HPV test (1).

Urine testing has been shown to compare favorably to physician-collected cervical sampling for detection of HPV infection, but to have less than optimal clinical sensitivity for the detection of high-grade cervical lesions by cytology or histology. Promising data were found among colposcopy patients in Oklahoma using first-void urine samples with the Trovagene test, with a high sensitivity for detecting 26 cases of CIN2+ (96%). In North Carolina (NC), we conducted a pilot study of colposcopy patients, finding testing of initial stream urine samples with Trovagene assay to be highly sensitive for detection of 11 CIN2+ cases (88%). In another NC pilot study, we found high sensitivity (90.9%) of the Onclarity assay in initial stream urine samples for the detection of 11 CIN2+ cases. While this preliminary research is highly promising, sample sizes restrict statistical power and have been limited to colposcopy patients. It is necessary to evaluate HPV urine testing with a greater number of CIN2+ cases in the screening population.

For primary screening (opportunities for all), there is substantial potential for the use of both HPV self-sampling and urine sampling to conduct large-scale screening of populations. For women found to be HPV-positive on either the self-sampling or urine sampling tests (selective screening), further research is ongoing to identify biomarkers to optimally triage strategies.

Methods
N/A

Results
N/A

Conclusion
N/A

References
Background / Objectives

Bhutan is strongly engaged in the prevention of cervical cancer. In the year 2000, the Ministry of Health launched a national cytology-based screening programme (Pap smear every three years for women aged 25–65 years) whose coverage ranges from 20% to 60% by district. In the year 2010, Bhutan was the first low/middle-income country (LMIC) to initiate a successful national vaccination programme against HPV with >90% coverage in girls age 12–18 years. In addition to monitoring the impact of HPV vaccination on HPV prevalence in the female population, IARC is engaged in demonstrating the feasibility and better effectiveness of HPV-based rather than cytology-based cervical screening, especially in rural settings.

Methods

Demonstration projects of HPV-based screening were carried out in the capital Thimphu and in rural areas (REACH-Bhutan). In REACH-Bhutan 3,648 women aged 30–60 years were invited and 2590 women (median age: 41 years) were enrolled in 15 Basic Health Units (BHU) differing in accessibility, size, and ethnic composition of the population. Self-collected samples were used and samples were tested by careHPV in Thimphu.

Results

Study participation in REACH-Bhutan was 71% (range by BHU: 31%–96%). Participation decreased with increase in age (81% in 30–39 year-old women but only 59% in ≥50 years), and travelling time. 50% of participants reported no previous screening, with the proportion of never-screened women varying significantly by BHU (range: 2%–72%). 265 women (10%; 95%CI 9%–11%) were careHPV-positive, with a significant variation by BHU (range: 5%–19%) and by number of sexual partners (prevalence ratio for ≥3 vs. 0–1=1.55; 95% CI: 1.05–2.27). HPV-prevalence in rural areas was slightly lower than that found in Thimphu in women of the same age group, i.e. 14.1% (95% CI: 12.0–16.4). Work-up of care-HPV+ women is ongoing.

Conclusion

Community-based cervical cancer screening, by testing self-collected samples for HPV, can achieve high coverage in rural Bhutan as shown in other LMICs. However, solutions to bring self-collection, HPV testing, and precancer treatment even closer to the remotest villages are needed.
References


WHAT IS THE TRADE-OFF BETWEEN REPRODUCTIVE AND ONCOLOGICAL OUTCOMES ACCORDING TO TREATMENT RADICALITY?

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Background / Objectives

The effectiveness of CIN treatments in preventing invasive cervical cancer has been suggested to be up to 99%, but several studies have shown the incidence of cervical cancer to still be elevated after treatment of CIN and the increased risk to last up to 20 years.

The mean age of women treated for CIN is very similar to with the mean age of first pregnancy in developed countries and the treatment of CIN has been associated with to an increased risk of preterm birth and perinatal morbidity in subsequent pregnancies. Furthermore, the radicality of the treatment has been shown to modify the effect of conization to reproductive morbidity.

Due to the ongoing debate regarding the risk and benefits associated with optimal treatment and follow-up strategies of CIN, our aim was to comprehensively assess the incidence of cervical and other cancers after treatment of CIN and the incidence of related reproductive morbidity by performing a series of systematic reviews and meta-analysis of the literature — regarding the invasive cancer incidence, and the reproductive morbidity after treatment of CIN correlated with the radicality of the treatment.

Methods

We searched electronic databases for studies reporting on cervical cancer incidence or mortality, preterm birth, and related outcomes after local, either ablative or excisional, treatment of CIN. Independent reviewers extracted the data and performed quality assessment for cancer incidence and reproductive morbidity outcomes separately. Pooled risk ratios were calculated with a random effects models. Inter-study heterogeneity was assessed with I² statistics.

Conclusion

Treatment of CIN increases the risk of subsequent preterm birth. Increasing radicality of the treatment, measured as the depth of the cone removed, is associated with higher risk of preterm birth. On the other hand, incidence of cervical cancer, as well as of other HPV-related cancers is increased after treatment of CIN despite a marked effect in cancer prevention.
To ensure optimal cancer preventive effect with minimal collateral harm, especially reproductive morbidity, we would need to compare the absolute risks and the effects the treatments have on them regarding both invasive cervical cancer incidence and preterm birth incidence — in different populations, and stratified according to radicality of the treatment.
CS 05-03
Effect of sex on oral HPV acquisition and persistence

G. D’souza

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Background / Objectives

This talk will review what is known about risk factors for oral HPV infection.

Methods

NA

Conclusion

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. Recent data will be reviewed which help to explain these differences in oral HPV infection between men and women.
Practical tips when counseling HPV discordant couples in session HPV infection: conciliate health and sexuality

M. Steben

Institut national de santé publique du Canada (Canada)

Background / Objectives

To review practical tips when counseling HPV discordant couples.

Methods

ICID has published a peer reviewed, evidenced based booklet about HPV testing reviewing the issues about 1) HPV testing and cervical cancer screening, 2) sexual transmission of HPV, 3) HPV vaccine as part of the pre or post-HPV test counseling, 4) HPV testing and the delivery of results and 5) complex psycho-social issues.

Results

HPV is the most common sexually transmitted infection in the world and most sexually active individuals will have an HPV infection at some point in their lives. Condom use for vaginal and anal intercourse offers some protection although it is incomplete. Most women having a pap test do not realize that in fact, we were already testing them for a complication of an STI without telling them! Self-blaming or partner-blaming might arise when a positive test is found. When counseling a couple, it is important to know if it is a new, an unstable or a stable long-term couple. Are questions about infidelity issues, sexual practices or cancer risk? Is it about emotional needs or information needs. Also, we need to know what part is leading to uncertainty, anxiety, sexual dysfunction or stress. It is also important to rapidly assess if the persons consulting you are concerned, anxious, hypochondriac or obsessive-compulsive. Discussing levels of risk can be quite difficult since people are seeking information. Discussion, counseling or therapy about emotional issues such as anxiety, guilt, negative anticipation, depression could be dealt by a psychologist knowledgeable about HPV issues. Discussion, counseling or therapy about sexual issues such as loss of sex drive, withdrawal, negative feelings about the partner, depression and sexual activities level of risk of transmission/acquisition could be dealt by a sex therapist knowledgeable about HPV issues. Issues of using barriers methods such as a condom or dental dam, prophylactic HPV vaccine use for the infected female and or her sexual partner, either male or female are also issues to be discussed during counseling sessions. Answering honestly about unknown such as the risk of recurrence and further risk at other sites may help compliance for the follow-up visits and time will help to answer some of the remaining questions.

Conclusion

HPV testing has the potential to greatly change the way we screen to prevent cervical cancer. Changing from looking for abnormal cells to looking for an STI has
the potential to harm fragile persons or relationships. Knowing practical points on how to counsel about these sensitive issues may alleviate the psycho-social burden of receiving a positive HPV test results.

References

Steben M, Kettner J, Medwick H, Ronald A, Wurtak G. Counselling Patients About HPV Testing, INTERNATIONAL CENTRE FOR INFECTIOUS DISEASES
Infection with one or more mucosotropic (species in the Alphapapillomavirus genus) HPVs is a near certainty for persons engaging in continued sexual activity over a lifetime. HPV is among the most successful sexually transmissible agents, having coevolved with human populations over many millennia. Primary prevention of HPV infection via behavioral modification and barrier methods is not as effective as for other sexually-transmitted infections, which are more prevalent in core risk groups. HPV infection is not so restricted; condom use provides only partial protection. On the other hand, prophylactic HPV vaccination has been surprisingly efficacious against acquisition of infections with specific genotypes. That HPV vaccination is effective in preventing transmission of HPV in heterosexual or homosexual couples in which one of the members has been vaccinated is difficult to demonstrate via randomized controlled trials. This may be a moot point, however, considering that there is now population-level evidence that vaccination leads to herd protection, which is a consequence of the reduction in overall transmission episodes in the population. Secondary prevention via case detection and treatment is not yet a realistic alternative or complementary solution because of the lack of effective treatment of asymptomatic HPV infections. The author will illustrate the challenges and directions on research on HPV transmission and show examples from a cohort study of heterosexual couples and randomized controlled trials of vaccination and microbicides.
Training and QA of colposcopy in Europe

P. Nieminen

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Background / Objectives

The performance of colposcopy is debatable. Many articles, especially from US, demonstrate poor sensitivity and specificity. On the other hand some, predominantly from UK, show it to be significantly better. Colposcopy could be compared to pathology or radiology, where the performance of the study is much dependent on the experience of the actor, i.e. here the colposcopists education and training. Still in many countries the incidence of cervical cancer has decreased significantly, thus colposcopy obviously has worked reasonably well, but the quality could be improved.

Methods

The European Federation for Colposcopy (EFC) has declared three colposcopy quality steps: education, training and practice. The education consists of recently developed and revised core curriculum, agreed standards for basic and advanced courses, EFC recognition of courses and provision of targeted courses. The training forms of defined training programme structure, caseload, assessment and electronic log-book. Not many European countries have their own training programmes, OBGYN training usually includes only the basics of colposcopy. Thus EFC is focusing to promote the subject and simultaneously trying to provide education, above all in forms of the core curriculum and colposcopy courses.

Results

What is a good colposcopist? The training should be recognized and preferably with a certification (and recertification). To keep the skills on acceptable level, continued medical education, caseload high enough and audit should be provided with proper intervals. To monitor the colposcopist's performance, EFC has identified few targets: Colposcopic examination prior to treatment should be done in 100% of cases, as well as the documentation of SCJ status. 85% of excisional treatment biopsies should have a CIN2+ in the lesion and 80 % clear margins in excisional treatment biopsies.

Conclusion

As a conclusion colposcopy should only be undertaken by trained colposcopists. There is a need for defined training programmes with QA and for agreed performance QA standards. The colposcopic practise needs also to be monitored.
CS 06-04
ASCUS LSIL normal colposcopy: the age factor

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Background / Objectives

Atypical cells of uncertain significance (ASCUS), or low grade squamous intraepithelial lesion (LSIL) smears, remain a challenge in cervical screening algorithms. These smear results may indicate the presence of high grade disease, but in the majority this is not the case, and many of the low grade lesions will clear by itself. In case of an ASCUS/LSIL smear and a normal colposcopy it remains uncertain whether further follow up is needed and if age plays a role in management.

This presentation will focus post colposcopy management of women with an ASCUS/LSIL smear with specific reference to age, and other markers.

Methods

The literature was searched regarding the follow-up of women with ASCUS/LSIL and normal colposcopy with specific regard to the effect of age on the incidence of CIN 2/3. Although studies have reported on the follow-up after ASCUS/LSIL and normal colposcopy, the relation with age has not been investigated specifically. As many other factors may play a more important role than age in the follow-up after ASCUS/LSIL, the literature was searched focusing on ASCUS/LSIL with normal colposcopy, and the use of additional markers during post colposcopy management.

Results

The largest cohort study reporting on follow up of women with ASCUS/LSIL and normal colposcopy is the ALTS trial. This trial showed that, after ASCUS/LSIL and colposcopy with/without biopsy indicating < CIN2 in 1836 women, a 10% risk of CIN 2+, and a 7% risk of CIN 3+, within 2 years of follow up remained. Age did not significantly influence these percentages but persistent hr-HPV detection was the strongest predictor, followed by follow up smear result en follow up colposcopic impression. Within the persistent hr-HPV positive women, a further risk stratification was possible with the highest risk in HPV 16 positive women followed by HPV 18 and 45. However, even among women who are HPV negative a 2-4% risk of CIN 3+ within 1-2 years follow-up was present.

Conclusion

In women with ASCUS/LSIL cytology and a subsequent colposcopy, excluding CIN 2+, there is an increased risk on CIN2+ during 2 years of follow up. hr-HPV persistence is the strongest predictor, especially HPV 16. Follow-up cytology and colposcopy are less strong predictors, while a small number of hr-HPV negative women are found with CIN 2+ during follow-up. These results indicate that women
with ASCUS/LSIL and colposcopy still need follow-up preferably with hr-HPV genotyping and cytology co-testing.

References


CS 07-01
Risk of CIN3 and cancer following conization for HG CIN, how to recognize patient at risk of recurrence

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Background / Objectives

The success of any cervical cancer screening program is best estimated by the impact of screening on cervical cancer rates. Cancer prevention is relatively unique to cervical cancer screening as compared to most other screened for cancers. To prevent cervical cancer one must identify and treat precancer. The efficacy of treatment is today, often balanced against the risk of adverse outcomes associated with treatment. In current practice in developed countries, treatment of precancer is effected by excision rather than ablation. The advantages of excision is that one can assess the completeness of the excision, correlate the pathology with prior specimens and detect occult carcinoma. This presentation will explore the literature based data that predict the risk of recurrence following excisional therapy.

Methods

The literature on the risk of recurrence after treatment, primarily short term risk will be defined via literature review.

Results

Historically, the primary indicator of recurrence risk was the status of the margins of the conization. However there are other variables that impact these estimates including the type of excision, size of the excision, how the specimen is processed, and distance of the precancer from the margin if the margin is negative. Whether the lesions is squamous or glandular is also important. Age and colposcopic adequacy are important factors as well. Currently all post-treatment follow-up strategies recommend cytology with or without HPV testing as an indicator of success of treatment. While even these assessments are confounded, the post-treatment samples may actually be superior to margin assessment of the excision specimen for predicting recurrence risk.

Conclusion

Clearly treatment works as documented by the success of the screening systems in most screening programs. But within the treated cohort, multiple factors interact to impact the short term success or failure of treatment as measured by short term recurrence rates. Accurate assessment can be highly confounded by several factors, mostly related to the size of the excision and the anatomy of the cervix. The historical utility of margin assessment is debatable in the era of electrosurgery.
Natural History of Anal HPV Infection and AIN in Young Women

A.B. Moscicki

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Background / Objectives

Although anal cancers in women are rarer than cervical cancers, anal HPV infections appear more common. Cumulative incidence of anal HPV reaches 70% over a 3 year period of time in young women. The true prevalence of anal HPV infection is unknown since sampling for anal HPV is usually contaminated by passing a swab through the perianal area. Hygiene habits (wiping front to back) may also result in anal sample contamination or a source of infection.

Methods

Literature was reviewed.

Results

As seen in the cervix, the majority of anal HPV infections clear within 3 years. HPV 16 appears to clear slower than the other hrHPV types which may explain its prominent role in anal cancer. Factors associated with anal HPV persistence include concomitant cervical HPV infection, alcohol use, smoking, anal finger sex, recent anal intercourse and no condom use during anal or vaginal intercourse.

Repeated anal HPV infections are extremely common. In one study, only one third of women were HPV-free after 3 years of observation. These anal HPV infections are not all benign. Although the data is sparse for general healthy populations, abnormal anal cytology occurs in approximately 4-6% of healthy women. The majority of lesions detected are benign (LSIL). Anal HSIL confirmed by histology is quite low—<1%. Risk factors for anal SIL appear similar to that of the cervix and include number of sex partners, reporting anal sex, smoking, history of abnormal cervical cytology and anal HPV infection.

The natural history of these lesions in healthy women is completely unknown. Progression in MSM has been estimated at 3% per year—if this translates to women who do not practice anal intercourse regularly or at all is questionable.

Conclusion

Although anal cancers occur more often in immunosuppressed women (i.e. HIV infected, solid organ transplants and those with autoimmune disease), the majority of anal cancers occur in women with no known immunodeficiency. There are no recommendations for anal cancer screening in general healthy populations. Screening is only recommended if symptoms of anal cancer are present.
ANAL CANCER IN HIV-POSITIVE AND HIV-NEGATIVE MEN AND WOMEN

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Background / Objectives

The natural history of high-risk human papillomavirus (HR-HPV) at anus is not fully illustrated, especially by HIV status.

Methods

We performed a systematic literature review and meta-analysis of HPV prevalence across the full spectrum of anal diagnosis. Data on 6,792 normal cytology, 3,918 low-grade lesions, 1,151 high-grade lesions and 2,098 invasive anal cancers were extracted from 77 studies with stratification of HIV status and gender as far as possible.

Results

Overall HPV prevalence increased with severity of anal lesion in men and women irrespective of HIV status. Among HPV-positive samples, HPV16 positivity increased with severity of anal lesion grade from 23-83% in HIV-negative and from 26-60% in HIV-positive men; from 13-87% in HIV-negative and from 17-75% in HIV-positive women. HPV16 less expressed in HIV-negative cancer compared to HIV-positive cases in men with prevalence ratio (PR) = 0.72 (95% CI: 0.63-0.82). Other HR-HPV types accounted for more important proportions of low- and high-grade lesions, but their contribution dropped in cancer. Only HPV16 accounted a greater proportion of HPV infection in cancer compared to normal cytology in HIV-negative (cancer:normal ratio = 5.00, 95% confidence interval [CI]: 4.41-5.67) and HIV-positive (cancer:normal ratio = 2.52, 95% CI: 2.21-2.88) individuals. Other non-HPV16 HR-HPV types were more prevalent in HIV-positive samples than HIV-negative counterparts across anal lesions in men and women.

Conclusion

HPV16 is uniquely carcinogenic at the anus, even in HIV-positive persons, and is the priority for cancer prevention.
Screening women for anal HSIL-- who and how to screen.

E. Stier

Boston University School of Medicine (United States of America)

Background / Objectives

The incidence of anal cancer is increasing in all women. Women at particularly increased risk of developing anal cancer include those living with HIV or have a history of non-anal HPV-related cancer. Detection of anal HSIL followed by treatment may prevent progression of anal HSIL to cancer. However, detection of anal HSIL can be difficult. There is not yet consensus on who should be screened and how that screening should be done.

Methods

Targeted screening has been suggested for women over age 35 who also have one of the following risk factors:

1) Infection with HIV
2) History of cervical, vaginal or vulvar cancer
3) History of CIN2+, VAIN2+ or VIN2+
4) Chronic immune suppression from other causes (e.g., organ transplantation, autoimmune disease)

Conclusion

Screening options include obtaining history of anal symptoms potentially related to anal cancer, digital anal rectal exam (DARE), anal cytology, anal HPV testing, and high resolution anoscopy (HRA) with directed biopsies. Anal cytology should not be used for screening unless HRA facilities are available.
SCREENING AND TREATMENT FOR ANAL HPV INFECTION AND ANAL HSIL IN WOMEN: WHO AND WHY

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Background / Objectives

The incidence of anal cancer in the general population is higher among women than men, and has been rising steadily since the 1970s. Although the incidence in the general population is relatively low, certain groups of women are at increased risk of anal cancer. As with algorithms designed to identify and treat cervical high-grade squamous intraepithelial lesions (HSIL) to prevent cervical cancer, anal cancer prevention programs include screening algorithms that include visual inspection of the at-risk areas, known as high resolution anoscopy (HRA). HRA requires substantial training and has limited availability, even in regions with large populations of at-risk individuals. Given the high cost and limited availability of HRA, several techniques have been used or are under investigation to screen at-risk individuals to identify those who should be referred for HRA.

Methods

The optimal approach to designing a rational anal cancer prevention program is to identify the populations that would most benefit from screening, identify optimal screening techniques, optimize treatment of anal HSIL, and document that treatment of anal HSIL reduces the incidence of anal cancer.

Results

The populations of women that would most benefit from screening are those with the highest risk of anal cancer, and these include women with a history of cervical or vulvar HSIL or cancer and women with immunocompromise due to HIV infection or other causes such as medication to prevent transplant rejection. Women with autoimmune diseases and other forms of immunocompromise may also be at increased risk. Anal cytology is the most commonly used screening tool to identify those who need HRA. Although it has a high positive predictive value for biopsy-proven HSIL when HSIL is found on cytology, it also has substantial limitations, including low sensitivity and routinely under-calls the grade of the lesion. Other techniques such as various forms of HPV detection are under investigation, either as primary screening tools or combined with cytology.

Conclusion

The utility of HPV as a screening tool alone or in combination with anal cytology needs to be established. However it is likely that the utility of HPV testing will vary considerably depending on the population being screened, e.g. HIV-infected men
who have sex with men versus HIV-uninfected women with a history of cervical HSIL, and each different at-risk population may need an individualized approach. The efficacy of treating HSIL to reduce the incidence of anal cancer is currently under investigation and will be key to establishing anal cancer prevention as standard of care for at-risk populations.
IMMUNOLOGY OF HPV DRIVEN OROPHARYNGEAL CANCER

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Background / Objectives

Human papilloma virus (HPV)-associated oropharyngeal squamous cell cancer (OPSCC) is a distinct clinical entity with a much better prognosis after (chemo)radiotherapy than HPV-negative OPSCC. We hypothesize that the virally-derived E6 and E7 antigens make HPV-associated OPSCC highly visible to the immune system, unleashing a strong antitumor response. To understand the constitution of an optimal antitumor response we have unraveled the immune contexture of OPSCC.

Methods

A comprehensive analysis by mass cytometry (CyTOF), flow cytometry and immunohistochemistry was performed on fresh and archived tissue of a cohort of 97 patients. In addition, the RNA-sequencing data of a cohort of 75 HPV16+ OPSCC patients present in the publicly available cancer genomic atlas (TCGA) database was used to analyze the tumor microenvironment by an analytical strategy to estimate subpopulations of tumor-infiltrating immune cells.

Results

CD4+ T cells formed the major component in OPSCC. Gene set enrichment analysis (GSEA) of the TCGA data in HPV16+ OPSCC with a high vs low CD4 gene expression revealed the enrichment of activated and effector memory CD4+ and CD8+ T cells as well as activated DC in HPV16+ OPSCC with a high expression of CD4. Indeed, flow- and mass-cytometry confirmed an increased percentage of DCs and highly activated effector memory CD4+ and CD8+ T cells in HPV16+ OPSCC. Furthermore, immunohistochemistry showed that these T cells were likely to produce interferon-gamma. Interestingly, the T-cell infiltrate of HPV16+ OPSCC comprised a population of cells expressing a specific C-type lectin receptor. The expression of this receptor strongly correlated to the overall survival of patients with HPV16+ OPSCC and was expressed on type 1 T cells recognizing the HPV oncoproteins E6 and E7.

Conclusion

OPSCC are infiltrated with HPV-specific T cells expressing a C-type lectin receptor that is also expressed by CD4+ T-cells dominating inflammatory diseases such as
rejections during graft versus host disease, hence a similar role may be expected in cancer and would be advantageous for tumor control.
PARADIGM OF HPV NATURAL HISTORY: FROM INFECTION TO CANCER

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Background / Objectives

Oral human papillomavirus (HPV) is a sexually transmitted infection that is etiologically responsible for the recent global increase in incidence of oropharyngeal cancer (OPC). The natural history of the progression from infection to cancer is not fully understood.

Methods

Current evidence indicates that HPV-driven OPC is preceded by oral infection with oncogenic HPV types, most commonly HPV16, by an estimated 10-30 years. Oral HPV infection is relatively rare, and is associated with sexual behaviors, male sex, and tobacco smoking. Although most infections are rapidly cleared, persistent infection is observed in some individuals, with increased risk among men and smokers. In a subset of individuals, oral HPV infection progresses to cancer. HPV-driven OPCs are frequently, but not always, characterized by integration of viral DNA into the host genome. Overexpression of HPV oncoproteins E6 and E7 with resultant disruption of p53 and Rb pathways is observed, among other genomic alterations that are still under investigation.

Conclusion

Future research to better characterize HPV-driven oncogenesis will carry important implications for the prevention, screening, diagnosis, and treatment of HPV-OPC.
HN 01-05
Combination immunotherapy with anti-PD-1 and therapeutic vaccines for HPV+ and HPV- Squamous cancers of the Head and Neck

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Background / Objectives

Squamous cancers of the head and neck are either caused by high risk Human Papilloma Virus (HPV), or by mutations promoted by smoking. Therapeutic vaccines directed against HPV, despite efficacy in pre-malignancy, do not generally mediate regression of invasive cancer. To test the hypothesis that the efficacy of vaccine-induced T cell responses may be amplified through immune checkpoint antibodies, we conducted a phase II trial of a synthetic long-peptide (SLP) HPV-16 vaccine (ISA101) and nivolumab, a PD-1 inhibitor, in pts with incurable HPV-16+ cancer.

Methods

Tumors were confirmed HPV-genotype 16. Patients were ECOG performance status 0-1 and had up to one prior regimen for recurrence. ISA101 100 mcgs/peptide was given Days 1, 22, 50. Nivolumab 3 mg/kg was given iv every 2 wks beginning day 8 for up to one year. Imaging was obtained baseline, 11 wks and every 6 wks thereafter. Baseline biopsies were mandatory. The primary objective was assessment of overall response rate (ORR) targeting 30%. Secondary objectives included tolerability, PFS, OS and HPV-specific immune response.

Results

The trial accrued 24 patients in one year; 22 with oropharynx cancer (OPC) and 1 pt each with anal and cervical cancer. Eighteen pts (75%) had progression within 6 mos of prior platinum and 1 was platinum-naïve. Twelve pts (50%) had prior cetuximab treatment. Study treatment was frontline for recurrence in 10/24 and second line in 14/24. ORR is 33% (8/24): 1 CR, 7 PR (1 unconfirmed), 3(13%) SD, 13 (54%) PD. ORR in OPC pts is 36% (8/22). Duration of response median 30.1+ wks (6- 49+ wks), 6/11 pts with PR/SD remain without progression. Of 8 pts with PR, 6 had progressed within 6 mos of prior platinum, 5 within 6 mos of prior cetuximab, and 5 were treated in second line. Median PFS is 2.7 mos and median OS is not reached with median follow up time among censored pts 8.6 mos. PFS rate at 6 mos: 33%, OS rate at 6 mos 74%.

Conclusion
The ORR of 36% in OPC pts compares favorably to the ORR of 16% for nivolumab monotherapy in p16+ OPC pts in Checkmate 141 (Ferris RL et al N Engl J Med 2016). These data suggest that the efficacy of vaccine-induced T cells can be augmented by anti-PD-1 therapy. Our findings should be confirmed in a larger randomized trial. In patients with HPV- H&N cancer we aim to apply a similar combination treatment, but the vaccine in that case will consist of a mix of SLP’s containing selected neo-epitopes generated by mutations in the cancer cells. The process of identifying optimal neo-epitopes and producing a personalized vaccine as well as the timelines involved will be discussed.
Background / Objectives

The fraction of cancer attributable to HPV is dominated by cervical cancer (83% of the 630,000 new cases of cancer per year worldwide). Three cancer sites in the head and neck (H&N) that are mainly due to tobacco and alcohol consumption have been also been associated with HPV: oropharynx (attributable fraction, AF= 13-60%, highest in Northern America and Europe) and, to a lesser extent, oral cavity and larynx (AF=1-4%).

Methods

Estimates of age (world) standardized incidence rates of HPV-associated cancer by country\(^1\). Comparison of HPV DNA prevalence in exfoliated cells from cancer-free tonsils and the oral cavity\(^2\).

Results

Globally, around 30% of oropharyngeal cancers (OPC) are caused by HPV (29,000 cases per year). For cancers of the oral cavity, 4,400 cases per year are attributed to HPV and for larynx, 3,800 cases. Approximately 80% of cases of H&N cancer attributable to HPV occur in men and their geographical distribution is diametrically different from that of cervical cancer showing a much higher burden in high-income than low/middle income (LMICs) countries. Countries with relatively high incidence rates of HPV-attributable H&N cancer (over 1.25 per 100,000) are located in Northern America and Europe. The remaining H&N cancers are due to tobacco smoking or chewing and alcohol. By comparison, cervical cancer incidence rates vary from <10 per 100,000 in high-income countries to >50 per 100,000 in LMICs mainly due to differences in the population prevalence of cervical HPV infection and in cervical cancer screening provision. On account of a greater predominance of HPV16 compared to cervical cancer, HPV 16 and 18 are globally responsible for 85% H&N cancer (vs 71% in the cervix).

Among adults in France, HPV prevalence was 3.6% in tonsil brushings and 13.1% in gargles, and HPV16 prevalence was 2.2% and 4.1%, respectively. Percent agreement in HPV detection in paired tonsil brushings and gargles in adults was 85.8% and positive agreement 9.5%. HPV prevalence in gargles significantly varied by sex (prevalence ratio in men vs women=2.1; 95% confidence interval; 1.1-4.1).

Conclusion
HPV-attributable H&N cancer differs from cancer of the cervix to many extents: 1) much lower incidence rates; 2) male predominance; 3) unfavourable trends in AF and incidence rates in some high-income countries concomitant to increases in HPV infection and the decline in tobacco smoking and; 4) inadequacy of cytology- or HPV-based screening. Gargle is not representative of HPV prevalence in the tonsil. HPV vaccination has currently the greatest potential for prevention of HPV-induced OPCs while the cessation of tobacco use is essential to avoid other H&N cancers.

References


PREVALENCE OF HUMAN PAPILLOMAVIRUS IN TONSILLAR/ADENOID TISSUE. A STUDY OF PARAFFIN-EMBEDDED ARCHIVAL MATERIAL FROM DIAGNOSTIC BIOBANKS IN NORWAY.

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Background / Objectives

HPV is found in an increasing number of tonsillar carcinomas. Thus, knowledge about the prevalence of viral infection in the oral cavity in the general population is of importance. Hypertrophic palatine tonsils are removed in all age groups and are suitable for epidemiologic studies. We examined the prevalence of HPV in benign tonsillar tissues removed in the period 1996 – 2014 at two Norwegian county hospitals, Akershus University Hospital (Ahus) and Vestfold Hospital (SiV).

Methods

Formalin-fixed and paraffin-embedded tonsils from patients ≤ 50 years were identified in the diagnostic archives from pathology departments at Ahus and SiV. DNA was isolated from all paraffin blocks, and from both palatines if present. 4 x 10 µm sections were cut from each paraffin block using standard procedures for preventing contamination. DNA was extracted according to standard procedures (Qiagen). The presence of HPV was investigated by using a modified GP5+/6+ PCR protocol, followed by hybridization of type-specific oligonucleotide probes coupled to fluorescence labeled polystyrene beads (Luminex suspension array technology), detecting and genotyping 37 HPV types (WHO validated protocol). Sample adequacy was evaluated through a beta-globin PCR.

Results

487 benign tonsillar samples were included. In the first 143 cases, HPV was identified in three samples (2,1%), two HPV16 and one HPV6. These samples were from patients aged 19 to 40 years and with a male/female ratio 2/1.

Conclusion

Preliminary data indicate a low HPV prevalence in cancer free tonsillar tissue. Data from all cases will be presented.
TIME TO CHANGE PERSPECTIVES ON HPV IN OROPHARYNGEAL CANCER - A SYSTEMATIC REVIEW OF HPV PREVALENCE PER OROPHARYNGEAL SUB-SITE

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Background / Objectives

Human papillomavirus (HPV) as a risk factor in oropharyngeal squamous cell carcinoma (OPSCC) is well established. However, accumulating data implies that the OPSCC concept is too broad and unspecific with regard to HPV prevalence and clinical importance. To further study the role of HPV in OPSCC by sub-site, a systematic review and meta-analysis of literature published 2013-2016 was performed.

Methods

A systematic review was performed using PubMed (January 2013–November 2016). Eligible studies included all studies that reported HPV data in both lymphoepithelial associated (i.e. tonsillar and base of tongue cancer; TSCC and BOTSCC respectively) and non-lymphoepithelial (i.e. walls of oropharynx, soft palate and uvula; “other” OPSCC) OPSCC. Pooled data by HPV detection method were analysed by calculating odds ratios, using a fixed effects model.

Results

Of the 1266 articles identified, 64 studies met the inclusion criteria, with 58 unique patient cohorts. HPV was more commonly found in TSCC and BOTSCC than in “other” OPSCC sub-sites. Total HPV prevalence in TSCC/BOTSCC was 56% (59% for TSCC only) as compared to 19% “other” OPSCC. Significant association of HPV to TSCC/BOTSCC vs. “other” OPSCC were observed no matter HPV detection method used, but statistical homogeneity was only observed when studies using algorithm based HPV detection were pooled.

Conclusion

HPV prevalence differs markedly between OPSCC sub-sites and while the role of HPV in TSCC/BOTSCC is strong, the role in “other” OPSCC is more uncertain and needs further evaluation before tailored treatment in this patient group.
INCREATING PREVALENCE OF HPV-POSITIVE TUMOR STATUS AMONG OLDER ADULTS WITH OROPHARYNGEAL CANCER, 1995-2013

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Background / Objectives

The incidence of oropharyngeal squamous cell carcinoma (OPSCC) is increasing among elderly adults in the United States [1]. The purpose of this study was to examine whether this recent rise in OPSCC among older age cohorts is due to increasing prevalence of human papillomavirus (HPV)-positive tumor status among elderly adults with OPSCC, and to determine whether HPV-positive tumor status continues to confer improved survival among older age cohorts.

Methods

This was a retrospective, multi-institutional study of OPSCCs diagnosed from 1995-2013. HPV tumor status was determined using immunohistochemistry for p16 overexpression and in-situ hybridization for HPV16 DNA and high-risk E6/E7 mRNA. Patient age at diagnosis, HPV tumor status, and calendar periods were compared. Survival was analyzed using Kaplan-Meier method and Cox Proportional Hazards models.

Results

There were 240 patients with OPSCC included in this analysis, of which 124 (52%) were HPV-positive. Between 1995-2013, median age increased among HPV-positive OPSCCs (ptrend=0.05), but not among HPV-negative OPSCCs (1995-2013 ptrend=0.86). The median age of HPV-positive OPSCC patients increased from 43.5 years (interquartile range [IQR] 52-60.5) in 1995-2000 to 52 years (IQR 56-53) in 2007-2013. Among patients ≥65 years old, the proportion of OPSCCs that were HPV-positive increased from 29% (5/17) in 1995-2000 to 52% (11/21) in 2001-2006, then 60% (12/20) in 2007-2013 (ptrend=0.07). This was similar to the increase noted among patients 55-64 years old (24% [5/12] in 1995-2000 to 58% [18/31] 2007-2013, ptrend=0.03). Among older age cohorts, HPV-positive tumor status was associated
with significantly improved overall survival compared with HPV-negative tumor status (>65 year old: HR=0.56, 95% CI=0.29-1.10; >60 year old HR=0.37 95%CI=0.20-0.67). The association of HPV tumor status with improved overall survival after OPSCC diagnosis was not significantly attenuated with increasing patient age (p=0.56 for interaction of age and HPV status).

Conclusion

The median age at diagnosis of HPV-OPSCC is increasing as the proportion of OPSCCs caused by HPV rises among older age cohorts. The favorable survival conferred by HPV-positive tumor status is not modified by age.

References

Background / Objectives

Oropharyngeal cancer (OPC) incidence is significantly increasing among men. Most cases are caused by human papillomavirus (HPV) and/or tobacco. As there are no proven methods for prevention or early detection, OPCs are diagnosed late (~85% at stages III/IV), requiring intensive chemo-radiation therapy which causes significant morbidity, life-long disabilities, and mortality. To increase patient survival and quality of life, biomarkers are needed to diagnose all OPCs at earlier stages. Viral and host (EPB41L3) gene methylation have been shown to predict cervical HPV infections that progress to CIN2/3 and recently were shown to be associated with AIN grade. We hypothesized that these methylation markers can also distinguish OPC from controls when measured in oral gargle specimens.

Methods

We conducted a case control study of 100 pre-treatment male OPC cases (base of tongue [n=49], tonsil [n=46], other OP [n=5]) receiving care at the Moffitt Cancer Center from 2014-2016. 100 disease free men were age and smoking history matched 1:1 to cases. Oral gargle specimens were collected from cases and controls and FFPE specimens from cases. Tumor and oral gargle extracted DNA was used in bisulfite conversion reactions using the EZ DNA methylation kit. Bisulfite modified DNA was purified and amplified by PCR primers. PCR was performed using the PyroMark PCR kit and products were captured by streptavidin beads in 96-well plates and pyrosequenced using PyroGold reagents. All runs included standard curves as positive controls of 0%, 50%, and 100% methylated human DNA and a non-template control.

Results

HPV 16 L1 and EB41L3 methylation was measurable in oral gargle specimens and discriminated cases from controls. Oral gargle HPV 16 L1 mean methylation was comparable to levels measured in tumor suggesting that the oral gargle represents the tumor (HPV 16 L1 mean methylation was 61.8 ± 27.4 in oral gargles vs 64.9 ± 25.4 in tumor). Among oral HPV 16+ cases 98.8% had detection of L1 methylation. In contrast, HPV 16 L1 was not methylated in the three oral HPV 16+ control
participants. *EBP41L3* methylation among controls was significantly lower than cases (0.99 ± 0.62 vs 2.59 ± 3.92, controls and cases, respectively). Significant differences in methylation levels by case status remained after stratifying HPV and p16 tumor status, indicating the biomarker detected both HPV-related and unrelated OPC cases. Importantly, oral gargle *EBP41L3* methylation distinguished early OPC stage (I/II) from controls (mean methylation 2.1 ± 1.5 vs 0.99 ± 0.6).

**Conclusion**

These data suggest that *EBP41L3* and HPV 16 L1 methylation measured in an oral gargle specimen may have utility as OPC early detection biomarkers.
HN 03-05
P16INK4A EXPRESSION PATTERNS PREDICT
CLINICAL OUTCOME OF PATIENTS WITH ORAL
DYSPLASIA IRRESPECTIVE OF HPV INFECTION
STATUS

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Background / Objectives

Diffuse p16INK4a overexpression is a surrogate biomarker of transforming HPV
infections in (pre-)cancerous lesions of the anogenital tract and in cancers of the
head and neck. p16INK4a overexpression has also been reported in a fraction of oral
premalignant lesions (OPL). Likewise, HPV DNA has been observed in a small
proportion of OPL. However, the significance of p16INK4a as a marker of a
transforming HPV infection in these precancerous oral lesions has not been
determined to date. Moreover, it is not clear how p16INK4a expression status may
impact on the lesion progression risk of OPL patients.

Methods

Formalin-fixed, paraffin-embedded (FFPE) tissue of mild, moderate and severe OPL
from the “Oral Cancer Prediction Longitudinal study” (OCPL), British Columbia,
Canada, were analyzed for HPV DNA, genotype and semi-quantitative viral load
applying Luminex technology. p16INK4a/Ki-67 immunohistochemistry was performed
on FFPE sections. p16INK4a expression patterns were classified as ‘negative’ (no
p16INK4a-positive cells), ‘focal/patchy’ (scattered p16INK4a-positive cells only) or
‘diffuse’ (p16INK4a-expressing cells in a clonal distribution). The results were further
correlated to lesion progression risk in the respective patients.

Results

HPV DNA from oncogenic types (16, 18 and 52) was detected in 15/241 OPL
samples (6.2%). Among 237 analyzable samples, 5.5% demonstrated a diffuse
p16INK4a expression pattern, 62.9% showed focal expression and in 31.6% no
p16INK4a expression was observed. A significantly higher association was observed
between HPV DNA-positivity and diffusely p16INK4a-stained lesions in comparison to
samples with a patchy expression pattern or no expression at all (p=0.04). However,
diffuse p16INK4a expression was also observed in a significant proportion of HPV
DNA-negative cases. Patients with focal p16INK4a expression had a significantly lower
risk of lesion progression over a total observation period of more than 12 years than OPL patients in which no p16\textsuperscript{INK4a} overexpression was observed at all (p=0.0001). Patients with diffuse p16\textsuperscript{INK4a} overexpression demonstrated an intermediate risk of lesion progression.

**Conclusion**

In contrast to the situation in the anogenital tract and in invasive head and neck cancers, diffuse p16\textsuperscript{INK4a} overexpression does not represent a specific biomarker of HPV-related precancerous lesions in the oral cavity. A focal p16\textsuperscript{INK4a} expression pattern in OPL provides valuable potential to identify cases with a low progression risk.
High-risk Human Papillomaviruses and p16 in Oral Cancer

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Background / Objectives

High-risk human papillomaviruses (HR-HPV), particularly types 16 and 18, play an important role in head and neck cancer, including oropharyngeal squamous cell carcinoma (OPSCC) and oral squamous cell carcinoma (OSCC). p16, a cell cycle inhibitor, has been postulated as a surrogate marker for HR-HPV, since p16 is aberrantly overexpressed, especially in HR-HPV-positive OPSCC. However, p16 as a surrogate marker for HR-HPV infection in cancers of the oral cavity remains controversial. The objectives of the study were to investigate the expression of p16 and the presence of HR-HPV in OSCC and oral verrucous carcinoma (VC) and to determine if p16 could be used as a surrogate marker for HR-HPV in OSCC and VC.

Methods

Forty one formalin-fixed, paraffin-embedded tissues of OSCC (n = 37), VC (n=4) with the clinical and histopathologic data of each case were collected. The expression of p16 was determined by means of an immunohistochemical technique. The staining intensity and numbers of the stained cells were scored and analyzed. The presence of HPV types 16 and 18 was detected by polymerase chain reaction (PCR). Descriptive statistics were employed to describe the demographic, clinical, and histopathologic parameters. The association between p16 overexpression, HR-HPV and all variables was determined by Fisher's exact test, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). In addition, the use of p16 as a surrogate marker for HR-HPV was analyzed by the sensitivity and specificity tests.

Results

p16 was overexpressed in 8/37 cases (21.6%) of OSCC and 2/4 cases (50%) of VC. HPV-16 was detected in 4/34 OSCC cases (11.8%) and HPV-18 was detected in 1/34 OSCC cases (2.9%). A co-infection of HPV-16/18 was detected in 1/4 VC cases (25%). Both p16 overexpression and HR-HPV were significantly associated with young patients with both OSCC and VC (p < 0.05, OR 20, 95% CI 1.9-211.8; p < 0.05, OR 23.3, 95% CI 2.4-229.7, respectively). p16 was able to predict the presence of HPV-16/18 in OSCC with 40% sensitivity and 79.3% specificity and in VC with 100% sensitivity and 66.7% specificity, respectively.
Conclusion

p16 overexpression was found in 24.4% of both OSCC and VC. HR-HPV, regardless of types, was detected in 15.8% in cases of OSCC and VC combined. The results of sensitivity and specificity tests suggest that p16 can be used as a surrogate marker for HR-HPV in OSCC and VC.

References


TARGETED SEQUENCING OF TONSILLAR AND BASE OF TONGUE CANCER AND HUMAN PAPILLOMAVIRUS POSITIVE UNKNOWN PRIMARY OF THE HEAD AND NECK REVEALS PROGNOSTIC EFFECTS OF MUTATED FGFR3

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Background / Objectives

Human papillomavirus positive (HPV+) tonsillar cancer (TSCC), base of tongue cancer (BOTSCC) and unknown primary cancer of the head and neck (HNCUP) have better outcome than corresponding HPV- cancers. To find predictive markers for response to treatment, and correlations and differences in mutated oncogenes and suppressor genes between HPV+ TSCC/BOTSCC and HPV+ HNCUP and HPV- TSCC/BOTSCC targeted next-generation sequencing was performed of frequently mutated regions in 50 cancer related genes.

Methods

DNA from 348 TSCC/BOTSCC and 20 HNCUP from patients diagnosed 2000-2011, was sequenced using the Ion AmpliSeq Cancer Hotspot Panel v2 to identify frequently mutated regions in 50 cancer related genes. Ion Torrent Variant Caller software was used to call variants.

Results

279 HPV+ TSCC/BOTSCC, 46 HPV- TSCC/BOTSCC and 19 HPV+ HNCUP samples qualified for further analysis. Mutations/tumor were fewer in HPV+ TSCC/BOTSCC and HNCUP, compared to HPV- tumors (0.92 vs. 1.32 vs. 1.68). Differences in mutation frequency of TP53 and PIK3CA were found between HPV+ TSCC/BOTSCC and HNCUP and HPV- TSCC/BOTSCC. In HPV+ TSCC/BOTSCC presence of FGFR3 mutations correlated to worse prognosis. Other correlations to survival within the groups were not disclosed.

Conclusion
In HPV+ TSCC/BOTSCC mutation of PIK3CA was most frequently observed, while TP53 mutations dominated in HPV- TSCC/BOTSCC. In HPV+ TSCC/ BOTSCC and HNCUP, mutations/tumor were similar in frequency and fewer compared to that in HPV- TSCC/BOTSCC. Notably, FGFR3 mutations in HPV+ TSCC/BOTSCC indicated worse prognosis.
HN 03-08
Continuing rise in oropharyngeal cancer in a high HPV prevalence area: A Danish population-based study from 2011-2014

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Background / Objectives

Human papillomavirus (HPV) is a critical element in the rising incidence of oropharyngeal squamous cell carcinoma (OPSCC) 1-3, although whether this trend will continue, and the types of HPV responsible, are currently unknown. We previously demonstrated an increased incidence of HPV-related OPSCC in the high HPV prevalence area of Eastern Denmark from 2000-2010 2,3. Therefore, we investigated if the incidence for OPSCC continued to rise, the association to HPV and putative HPV types in Eastern Denmark from 2011-14. We then projected the expected incidence of OPSCC versus cervical cancer through to 2020.

Methods

Patients with OPSCC (tonsillar squamous cell carcinoma (TSCC) and base of tongue squamous cell carcinoma (BSCC)) were identified via the Danish Head and Neck Cancer Group and the Danish Pathology Databank (n=700). Tumors were re-reviewed and assessed using p16 immunohistochemistry, HPV DNA PCR, with genotyping by next generation sequencing.
Results

Sixty-two % (432/700) of tumors were HPV-positive (HPV+). The total incidence rate (pr. 100,000) for OPSCC increased from 4.0 in 2011 to 4.5 in 2014, primarily due to a rise in HPV+ tonsillar squamous cell carcinomas (TSCCs) and HPV+ base of tongue squamous cell carcinomas (BSCCs), although numbers of HPV-negative (HPV-) OPSCC also increased during the study period. The majority of HPV+ tumors were HPV16 DNA positive (86%), but we also identified HPV33 DNA (6%), HPV35 DNA (4%), and others (3%), including HPV18, 26, 31, 45, 56, 58, 59, and HPV67.

Conclusion

An increasing incidence of OPSCC is driven primarily by HPV+ OPSCC. Sixty-two % of tumors were HPV+, which is a high prevalence, although the lower number of HPV- cases has yet to stabilize. HPV16 was the predominant genotype, although a significant proportion (14%) was of another genotype. Our projections suggest that the number of HPV+ OPSCC will exceed that of cervical cancer in 2016 in Eastern Denmark.

References


Efficacy of AS04-Adjuvanted HPV-16/18 Vaccine in Reducing Oropharyngeal HPV Infections in Adolescent Girls – Results from a Community-Randomized Trial

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Background / Objectives

Aside from its causal association with cervical cancer, high-risk Human Papillomavirus (HPV) infections can lead to the development of oropharyngeal cancers. Over the last two decades, prevalence and incidence of high-risk HPV-positive oropharyngeal cancers in non-vaccinated populations has dramatically increased in affluent countries. Prophylactic HPV vaccines have already been shown to be highly efficacious in preventing persistent cervical infections and precancerous lesions associated with the most prevalent carcinogenic HPV types (HPV-16 and -18) and some related oncogenic HPV types.

We present here secondary endpoint results of a phase III/IV, community-randomized, controlled study (NCT 00534638) evaluating vaccine efficacy (VE) of the AS04-adjuvanted HPV-16/18 vaccine (AS04-HPV-16/18) against oncogenic HPV oropharyngeal infections in adolescent girls.

Methods

From 2007 to 2010, 80,272 adolescents aged 12-15 years from 33 randomized communities in Finland were invited to participate in the study. 22,444 girls and 11,968 boys were allocated to 3 arms (A, B, C) of 11 communities each. Vaccinated subjects received either AS04-HPV-16/18 or hepatitis B virus (HBV) vaccine at months 0-1-6.

Oropharyngeal samples were collected from girls born in 1994-95 after age 18.5 years and before age 19 years (5-6 years after vaccination). HPV DNA prevalence in the oropharyngeal samples was determined by SPF-10 line probe assay (LiPA) and Multiplex Type-specific PCR.

VE was defined as a relative reduction of oropharyngeal HPV prevalence among HPV-vaccinated pooled-arms A and B girls compared to all HPV non-vaccinated girls from arm C (control arm).

Results

In arms A and B, 89.5% (8,235/9,203) of vaccinated girls and boys, and 89.6% (6,601/7,367) of vaccinated girls respectively, were blinded and received AS04-HPV-
16/18. Other vaccinated participants in arms A and B (6,614) and all vaccinated subjects (10,724) in arm C received HBV vaccine.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Arm</th>
<th>N</th>
<th>n</th>
<th>VE (%)</th>
<th>95% CI (LL – UL)</th>
<th>p-value</th>
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<tr>
<td>16/18</td>
<td>A &amp; B</td>
<td>3,192</td>
<td>9</td>
<td>82.4</td>
<td>47.3 – 94.1</td>
<td>0.002</td>
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<td></td>
<td>C</td>
<td>1,679</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31/45</td>
<td>A &amp; B</td>
<td>3,192</td>
<td>3</td>
<td>75.3</td>
<td>12.7 – 93.0</td>
<td>0.030</td>
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<tr>
<td></td>
<td>C</td>
<td>1,679</td>
<td>9</td>
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<td>3,192</td>
<td>9</td>
<td>69.9</td>
<td>29.6 – 87.1</td>
<td>0.006</td>
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<tr>
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<td>C</td>
<td>1,679</td>
<td>16</td>
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</tbody>
</table>

CI: confidence interval; LL: lower limit; N: number of subjects; n: number of positive samples; UL: upper limit; VE (%): vaccine efficacy (1-Odd Ratio)

Conclusion

AS04-HPV-16/18 shows evidence of high VE against oropharyngeal infections with vaccine HPV types (HPV-16/18) and other oncogenic HPV types (HPV-31/33/45) in adolescent girls vaccinated at the age of 12-15 years.

Funding: GlaxoSmithKline SA

* Authorship on behalf of the HPV-040 study group
FOUR-PARAMETER MODEL FOR PREDICTING OUTCOME IN PATIENTS WITH HPV-POSITIVE TONSILLAR AND BASE OF TONGUE SQUAMOUS CELL CARCINOMA


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Background / Objectives

Therapy in head and neck cancer has become intensified, leading to improved survival but also increasing side effects. Patients with HPV-positive head and neck cancer generally have a very good prognosis, with 80% 3-year disease-free survival after treatment with radiotherapy alone. Thus, we aimed to construct a model, based on biomarkers and clinical data, that can accurately predict prognosis in patients with HPV-positive TSCC/BOTSCC (tonsillar/base of tongue squamous cell carcinoma) in order to be able to select patients good prognosis for milder treatment, decreasing side-effects without risking relapse.

Methods

258 patients with HPV16 DNA and E7 mRNA-positive TSCC/BOTSCC, treated curatively between 2000-2011, were included. Candidate descriptors were: age, T-stage, N-stage, clinical stage, CD8 TIL numbers, tumour location, HPV16 E2 and E5 mRNA expression, HLA class I staining and type of treatment. The outcome was 3-year progression-free survival. Patients were split randomly 65/35 (168/90) into training and validation sets, and LASSO regression with 10-fold cross-validation was used to select a model in the training set, whereafter model performance was evaluated in the validation set.

Results

None of the intensified treatment options (accelerated radiotherapy, chemoradiotherapy, brachytherapy or cetuximab) improved survival compared to conventional radiotherapy. High numbers of CD8 TILs and age<60 were the strongest predictors of survival, followed by T-stage<3 and HPV16 E2 mRNA expression. A model where the presence of three of four of these markers defined good prognosis had 56% sensitivity and 98% positive predictive value (non-relapse defined as event). The model identified 35% of our cohort as overtreated patients who would have benefitted from de-escalated therapy.

Conclusion
We built a model for patient stratification based on age, T-stage, CD8 TILs and HPV16 E2 mRNA that has very high accuracy in selecting patients that survive. Patients fulfilling model criteria for good prognosis should receive only conventional radiotherapy in order to limit side-effects.
Background / Objectives

The current controversial discussion of the link between disease-specific survival of HPV positive and negative patients with head and neck squamous cell carcinoma (HNSCC) was crucial in carrying out this meta-analysis.

Our experimental study on HPV diagnostics on oral squamous cell carcinoma (OSCC) showed that unequal, inhomogeneous patient collectives are compared with each other in the assessment of the influence of the HPV status on the survival of patients (1). Furthermore, insufficient detection methods of HPV are still used. A false interpretation of survival rates and the impact of HPV is the consequence.

Methods

170 studies from 2007 to 2014 (NCBI / Pubmed) were evaluated in this meta-analysis. Exclusion criteria were patient groups with n <70. The HPV detection method, patient characteristics (age, sex), tumor location and stadia, (neo-) adjuvant therapy measures and survival times were recorded and evaluated.

Conclusion

The analysis showed that the survival times in many studies were not evaluated in a multifactorial manner, since important confounders were ignored. The analyzed HPV detection methods were often not sufficient to represent HPV infection. Moreover, in a variety of studies, oropharyngeal and oral squamous cell carcinomas were evaluated as one cohort. With regard to the different survival rates of the affected patients of these cancers of different anatomical regions a subdivision of anatomical regions is indispensable. Studies of OSCC, that took care about the latter points did not show any improved survival of the HPV-positive collectives.

The results of the experimental study of our research group were confirmed by our metaanalysis (1). The discussion about a revision of therapy strategies of OSCC dependent of the HPV status must be rejected according to current knowledge. Studies published so far have to be questioned very critically and are not a sufficient for therapeutic decisions.

References

(1) Impact of HPV infection on oral squamous cell carcinoma.
Götz C, Drecoll E, Straub M, Bissinger O, Wolff KD, Kolk A.

MICROBIOTA ASSOCIATED WITH ORAL CANCER – A POTENTIAL DIAGNOSTIC AND PROGNOSTIC MARKER

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Background / Objectives

In 2012, over 300,000 new cases of patients suffering from the oral cavity and lip cancer were diagnosed worldwide. Smoking and the use of alcohol are the main risk factors of oral cancer explaining nearly 80% of all cases. In addition to human papillomavirus (HPV), oral bacteria have recently been implicated in the pathogenesis of oral cancer, particularly squamous cell carcinoma. Periodontal disease (gum disease) may be causally related to oral cancer through inducing chronic inflammation. The oral bacterial microbiota consists of 500-700 species and specific oral bacteria such as Porphyromonas gingivalis and Fusobacterium nucleatum, have been shown to have oral carcinogenic potential in vitro and in animal studies. Furthermore, it is well established that certain oral bacteria express the enzyme alcohol dehydrogenase which catalyses the production of acetaldehyde, the first metabolite of ethanol metabolism and a grade I carcinogen associating with the use of alcoholic beverages and/or smoking. Assessing the oral microbiota may therefore carry potential as a diagnostic and prognostic marker in oral cancer (Guerrero-Preston et al., 2016).

Methods

Describing oral bacterial colonization and how oral microorganisms interact with each other and the host are most likely the key determinants for understanding disease etiology and progression. Future work should strive for methodological standardization including acquisition, storage and handling of samples. It is also important to recognize that the oral cavity and the head and neck region represent areas with various tissue specific functions and diseases. Case-control studies implementing full metagenomics or metatranscriptomics analyses should be undertaken in an attempt to explain the role of the oral microbiota in oral cancer. Large-scale, longitudinal studies with follow-up are needed preferably commencing with potentially malignant oral lesions, which precede actual carcinoma in most cases of oral cancer.

Conclusion

It is paramount to include the known major determinants of oral disease risk in the analyses assessing the contribution of oral microbiota to disease development. Tooth decay, periodontitis, oral hygiene in general, diet, smoking, and alcohol consumption have all been reported to affect oral microbiota composition. It is intriguing to
hypothesize that altered microbiota may be one of the mechanisms by which these risk factors lead to carcinoma.

References

THE ROLE OF SEROLOGICAL MARKERS

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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 and ongoing 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 Luminex-based 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

In multiple studies, antibodies to HPV16 E6 were shown to be the serological marker most strongly associated with OPC. They were almost exclusively present in cases with molecularly defined HPV-driven OPC, yielding a sensitivity and specificity compared to tumor HPV status exceeding 90% and 95%, respectively. The prevalence of HPV16 E6 antibodies in healthy controls has been repeatedly shown to be in the range of 0.5%, i.e. the disease 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, have been repeatedly shown in both case-control and prospective cohort studies to be highly specific biomarkers for detection and prediction of OPC. To date, the trigger for seroconversion (e.g., yet to be described premalignant OPC lesions) is not understood, and the clinical implications of early HPV-OPC detection are under debate.
The use of different biomarkers for predicting clinical outcome in human papillomavirus positive tonsillar and base of tongue cancer


Karolinska Institutet (Sweden)

Background / Objectives

Head-neck cancer therapy has become more aggressive, with the addition of chemotherapy and EGFR inhibitors to previously administrated radiotherapy. With radiotherapy alone, 3-year disease-free survival (DFS) is 80% for HPV-positive tonsillar and base of tongue cancer and even better for patients with favorable characteristics and biomarkers, suggesting therapy could be de-escalated for some patients, decreasing side-effects. For this purpose we combined several biomarkers and built a model to predict progression-free survival for patients with HPV-positive tonsillar and base of tongue cancer.

Methods

Patients with tonsillar and base of tongue cancer treated curatively between 2000-2011, with HPV16 DNA/E7 mRNA positive cancers also examined for CD8+TILs, HPV16 mRNA and HLA class I expression were included in the study. Patients were split randomly 65/35 into training and validation sets, and LASSO regression was used to select a model in the training set. Thereafter, the performance of the model was evaluated in the validation set.

Results

In total, 258 patients with HPV DNA/E7 mRNA positive tumors were included in the study with 168 patients in the training set and 90 patients in the validation sets. No treatment improved survival in comparison to radiotherapy alone. CD8+ TIL counts and young age were the strongest predictors of survival. They were followed by T-stage <3 and presence of HPV16 E2 mRNA. The model had an area under curve (AUC) of 76%. Furthermore, a model where the presence of three of four of these markers defined good prognosis captured 56% of non-relapsing patients with a positive predictive value of 98% in the validation set.

Conclusion

Combined together, CD8+ TIL counts, age, T-stage and E2 mRNA expression could predict progression-free survival for 56% of the patients with very high probability.
This model could be useful for identifying patients that could be offered the possibility of randomized trials with milder treatment, with less side effects, without worsening prognosis.

References

Quality of life following HPV driven OPC

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Background / Objectives

This talk will review quality of life issues among survivors with an HPV-related oropharyngeal cancer (HPV-OPC).

Methods

NA

Conclusion

We will discuss issues of anxiety, relationship dynamics, emotional aspects of cancer, and knowledge and unanswered questions or concerns around HPV among survivors and their families.

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Background / Objectives

Oropharyngeal cancer has emerged in the last three decades as an important HPV-associated head and neck cancer and is projected to surpass cervical cancer as the dominant HPV-related cancer in the United States by 2020. While it is generally confers survival advantage compared to non-HPV related head and neck cancer, there is limited data on the trends in oropharyngeal cancer survival since the recognition of the role of HPV in head and neck cancer. The objective of the study was to describe trends in relative survival of oropharyngeal cancer in the United States in the last three decades.

Methods

Patients diagnosed with index oropharyngeal cancer between 1975 and 2009 were abstracted from the Surveillance, Epidemiology, and End Results (SEER) 9 database to determine their 5-year relative survival percentage. Joinpoint regression analyses determined annual percent change (APC) trends in 5-year survival by year of diagnosis stratified by race, sex, and age. APCs were conducted at an alpha of 0.05 and were two-tailed.

Results

There were 25,034 patients with oropharyngeal cancer included in the analyses. Seventy-three percent (73%) of participants were male, and 83% were white. Overall, relative survival from oropharyngeal cancer increased from 33% in 1975 to 68% in 2009, yielding 106% increase in survival over the 35-year period of the study. Specifically, relative survival increased from 1975-1988 (APC=1.34, p <.05) and then more sharply increased from 1988-2009 (APC=2.67, p <.05). Both blacks and whites exhibited steady relative survival increases from 1975-2009, however whites had overall higher survival rate increases than blacks (whites APC=2.31, blacks APC=2.17, p <.05). Comparing males vs. females, relative survival among males and females also increased over the study period with males having an overall better survival than females (females APC=1.21, males APC=3.76, p <.05). This better survival rates among males was largely due to the sharp increase in survival among males from 1991-2003 (APC=3.76, p <.05). After 2000, males tended to have better survival than females. There was also increase in survival across age-groups (20-44,
45-64, and 65+ years), with a marked recent increase among 65+ years (2001-2009 APC=4.27, p <.05). However, the 20-44 year group had the best survival rates as expected.

**Conclusion**

Our study show that there has been significant increases in oropharyngeal cancer relative survival from 1975-2009 across races, sexes, and ages. Patients diagnosed at younger ages had better survival compared to older patients. The fact that whites had better overall survival rates than blacks may point to the existing health disparities issues in the United States.
What is the ideal HPV screening method in the oropharyngeal region? SHIO Study


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Background / Objectives

Over the past decade, there has been an increase in the number of instances of head and neck squamous cell carcinoma (HNSCC) cases reported in parallel with HPV (human papillomavirus) infection. The strongest ratio of HPV to HNSCC has been found in oropharyngeal SCC (OPCC), especially in the tonsils. Hypothetically if the HPV virus is caught early in the background, then potentially SCC could be prevented. The big question is how it can be prevented and screened? The purpose of SHIO (Screening of HPV infection in oropharyngeal region) study is to determine an effective HPV screening method that can be easily and quickly applied, and can therefore become the benchmark or gold standard for HPV testing worldwide. The study will also assess with a view to estimating the HPV incidence of the HN region in Hungarian adults.

Methods

All patients undergoing routine tonsillectomy for benign indications in the Hospital were invited to participate in our study. Using a questionnaire, all individuals were interviewed about their sexual behavior, socioeconomic factors, drug use and other known or suspected HPV infection risk factors. During this study three different samples were taken for HPV examination before tonsillectomy (1. rinse of mouth and tonsil area, 2. cytobrush sample from surface of tonsils, 3. sampling from tonsils with cytobrush after exerting pressure), and following tonsillectomy a fourth sample was then taken from tonsils for HPV testing (Anyplex™ II HPV28 Detection, Seegene Inc., Seoul, Korea) and a histological examination of the tonsils followed. Before the main project, we performed a pilot trial involving the inclusion of 100 patients, but in order to get statistically powered results, a sample-size of approximately 900 patients is required for the full trial to be meaningful. To achieve this number of patients we plan to cooperate with other hospitals.

Conclusion
Based on our experiments the Anyplex™ II HPV28 Detection test can be used successfully in the examinations of HN samples. In terms of sexual habits, the samples proved to be representative. During the pilot we have set up a well-established sampling and screening system designed between the hospitals, the clinic and the laboratory. Now we are ready for the complete study. With more complete studies we will be able to build an accurate epidemiology of HPV infection within Hungarian adults using a non invasive screening method in the HN region.
Molecular targeting of the DNA damage response as a novel approach to deintensify the therapy of HPV-positive HNSCC

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Background / Objectives

Clinical data demonstrate an enhanced radiation sensitivity of HPV+ HNSCC, a feature also observed on the cellular level in HPV+ HNSCC cell lines. For the latter we could show that the underlying mechanism is a defect in DNA double-strand break repair associated with a profound and sustained arrest in G2 (Rieckmann 2013). Specific inhibitors of central components of the DNA damage response (DDR), such as PARP1, Wee1 and Chk1 are being tested in clinical trials in HNSCC and the intrinsic DNA repair defect of HPV+ HNSCC cells may render these tumors especially susceptible for further radiosensitization.

Methods

Mechanistic proof of efficacy of the various inhibitors was performed using Western blot, immunofluorescence microscopy and assessment of cell cycle distribution. DDR-Inhibitors: PARP – Olaparib; Chk1 – PF00477736, LY2603618, Prexasertib; Wee1 – AZD-1775. Standard therapeutics: Cetuximab, cisplatin. Radiosensitization was assessed using colony formation assay. HPV+ HNSCC cells: UT-SCC-45, 93-VU-147T, UD-SCC-2, UM-SCC-47, UPCI-SCC-154.

Results

While the inhibition of EGFR by cetuximab is being extensively tested for HPV+ HNSCC in phase 3 clinical trials, on the cellular level cetuximab completely failed to exert a meaningful cytotoxic effect or radiosensitization of any of the 5 HPV+ HNSCC strains tested (Güster 2014). In contrast, the inhibition of Chk1 interfered with the radiation-induced G2-arrest and resulted in radiosensitization in all HPV+ HNSCC cell lines, as well as the targeting of DNA repair processes through the inhibition of PARP1 (Busch 2013, Güster 2014, Busch 2017). Targeting Wee1 resulted in an accumulation of the HPV+ cells in the S-phase rather than in the intended release from the radiation-induced G2-arrest and it induced a compensatory activation of Chk1. The combined inhibition of Wee1 and Chk1, however, was already effective using massively reduced doses of both inhibitors and resulted in efficient radiosensitization (Busch 2017). In all cases the radiosensitizing effect was far stronger in the HPV+ HNSCC strains than in normal human fibroblasts used as a surrogate of p53-proficient normal tissue cells.

Conclusion
While the inhibition of EGFR fails to confer radiosensitization of HPV+ HNSCC on the cellular level, the inhibition of the DNA damage response was found to be effective. Our data strongly suggest that these targeting approaches further interfere with the ability of HPV+ HNSCC cells to cope with radiation-induced DNA damage and may represent viable options for the deintensification of therapy. The verification of these results in independent patient derived xenograft models as a next step towards a clinical use is currently being planned.

References

Rieckmann et al. 2013. HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. Radiother Oncol. May;107(2):242-6

Güster et al. 2014. The inhibition of PARP but not EGFR results in the radiosensitization of HPV/p16-positive HNSCC cell lines. Radiother Oncol. Dec;113(3):345-51

Busch et al. 2013. HPV-positive HNSCC cell lines but not primary human fibroblasts are radiosensitized by the inhibition of Chk1. Radiother Oncol. Sep;108(3):495-9

Busch et al. 2017. G2-checkpoint targeting and radiosensitization of HPV/p16-positive HNSCC cells through the inhibition of Chk1 and Wee1. Radiother Oncol. Feb;122(2):260-266
Simultaneous Quantification of HPV Oncogene (E6,E7) mRNA and PD-L1 Protein Expression in Oral Cancer Samples Using Flow Cytometry

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Background / Objectives

Human papillomaviruses are a family of DNA viruses that infect the epithelium leading to the formation of lesions with the ability to progress into carcinoma in many forms. Research into causality of head and neck squamous cell carcinoma (HNSCC) have found a link to HPV infections affiliated with better survival than tobacco associated HNSCC. In addition, advances in immuno-oncology have brought immunotherapy to the forefront of cancer treatment across cancer types. Anti-PD-1 and anti-PD-L1 therapies are being explored for their efficacy in treatment of head and neck cancers. Here we present data that demonstrates a combined diagnostic approach to quantify both HPV E6,E7 and PD-L1, two markers important in the management of head and neck cancer.

Methods

Samples were collected from Institut Gustave Roussy patients with lesions of the oral pharynx. Swabs were collected and placed into a collection vial with a proprietary fixation/permeabilization solution (IncellCollect, IncellDx, Inc.) and shipped overnight on cold packs for processing. Upon receipt, samples were passed through a 35 uM filter to remove aggregates. Cells underwent RNA in situ hybridization with E6, E7 mRNA probes (HPV OncoTect) , were labeled with PD-L1 Ab (clone 28-8), and then stained with a cell cycle dye identify single nucleated cells, and to analyze cell cycle prior to analysis on the flow cytometer (CytoFLEX, Beckman Coulter, Inc).

Results

We analyzed samples from 20 patients with oral cancer with the combined E6, E7 mRNA/PD-L1 protein assay by flow cytometry. The percentage of cells expressing E6,E7 mRNA and the percentage of cells expressing PD-L1 was calculated. E6,E7 result was compared to a second HPV test method as well as biopsy result. The study is ongoing and data will be presented at the meeting.

Conclusion

We report a novel flow cytometric assay to quantify both HPV E6, E7 mRNA and PD-L1 simultaneously in single cells from head and neck squamous cell carcinoma. The
ability to characterize both markers in one test can provide clinicians with insight into the prognosis of the patient based on their HPV mRNA status and PD-L1 expression.
HN 06-05
Influence of HPV-status on survival of patients with tonsillar squamous cell carcinomas (TSCC) treated by surgery - a 10 year retrospective single centre study

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Background / Objectives

The positive prognostic value of HPV-infections in oropharyngeal squamous cell cancer (OSCC) patients has led to the initiation of prospective clinical trials testing the value of treatment de-escalation. It is unclear how to define patients potentially benefiting from de-escalated treatment, whether a positive smoking history impacts survival data and what kind of de-escalation might be best. Here, we investigate the effect of HPV-status, smoking habit and treatment design on overall survival (OS) and progression free survival (PFS) of 126 patients with tonsillar SCC who underwent CO2-laser-surgery and risk adapted adjuvant treatment.

Methods

HPV-DNA-, HPV-mRNA-, and p16INK4A-expression were analyzed and results were correlated to OS and PFS. Factors tested for prognostic value included HPV-status, p16INK4A-protein expression, therapy and smoking habit. Log rank test and p-values ≤0.05 defined significant differences between groups.

Results

The highest accuracy of data with highest significance in this study is given when the HPV-RNA-status is considered. Using p16INK4A-expression alone or in combination with HPV-DNA-status, would have misclassified 23 and 7 patients, respectively. Smoking fully abrogates the positive impact of HPV-infection in TSCC on survival. Non-smoking HPV-positive TSCC patients show 10-year OS of 100% and 90.9% PFS when treated with adjuvant RCT.

Conclusion

In conclusion, the presented data show that high-precision HPV-detection methods are needed, specifically when treatment decisions are based on the results. Furthermore, smoking habit should be included in all studies and clinical trials testing HPV-associated survival. Adjuvant RCT especially for HPV-positive non-smokers may help to avoid distant failure.
Background / Objectives

Oropharyngeal squamous cell carcinomas (OSCCs) are increasing in incidence and are associated with considerable morbidity and mortality. Recognised risk factors include sexual behaviour, high risk oral HPV infection and poor oral hygiene. Large proportions of the general population attend dental clinics on a regular basis, where they routinely undergo assessment of their oral hygiene. We set out to investigate whether risk assessment for OPSCC could be undertaken in two general dental clinics.

Methods

Patients aged ≥18 years attending two general dental clinics in Sydney, Australia were invited to participate in the study. Participants completed demographic and behavioural questionnaires. Oral Hygiene Index (OHI) was recorded by examining six tooth surfaces separately for debris and calculus status. A score ranging from 0 to 3 for each examined surface was recorded, and the OHI calculated using a formula. 10ml saline oral rinses were obtained prior to their booked dental procedure, which were subsequently tested for Human Beta Globin gene (HBG) and HPV genotypes by PCR.

Results

Three hundred and nine dental patients were approached and 302 (97.7%) agreed to participate. Two (0.7%) people were excluded because of inadequate sampling. The mean age of the 300 remaining participants was 48 years (range 19-87), of whom 131 (43.6%) were male, 12 (9.2%) of men reported a history of sexually transmitted infections and one (0.3%) was known to be HIV infected.

HBG was detected in all samples and HPV genotypes in six samples (2.0%), all men. The HPV genotypes were one each of types 16, 66, 51, 35, 58 in five participants, and 18 combined with 52 in one participant. Eighty nine (67.9%) of men reported oral sex practices. Four (4.5%) of these 89 had oral HPV detected (all exclusively with female partners). Five (2.1%) of the 233 participants with an OHI of ≤ 1 (good oral hygiene), compared to one (4.3%) of the 23 with an OHI ≥ 2 (poor oral hygiene) had at least one HPV genotype detected.
Conclusion

A large majority of patients approached at the general dental clinics agreed to participate. The clinical relationship between the dental practitioner and patients enabled the collection of potentially sensitive demographic and behavioural data. Oral hygiene assessment could be performed accurately and professionally. Oral rinse sampling was well tolerated by participants, and satisfactory samples were obtained. Dental surgeries may be ideally placed to undertake risk evaluation for OPSCC. Larger trials are needed to evaluate this method further, and determine correlations between persistent oral HPV infection and risk.

References

1. Bui TC. Gum disease; poor oral health linked to cancer-causing oral HPV infection. Obesity, Fitness and Wellness Week, 7 September 2013:


Juvenile-Onset Recurrent Respiratory Papillomatosis : a french 43 cases serie

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Background / Objectives

Finding appropriate treatment to HPV infections is one of today’s scientific challenges. Amongst head and neck HPV-related pathologies, the Juvenile-Onset Recurrent Respiratory Papillomatosis (JORRP) might be the least understood of all. It is currently treated with repeated tumor-removal surgeries along with adjuvant therapies which can lead to severe side effects and a certain cost. Due to the scarcity of the JORRP, few studies are accurate in assessing its natural history. National registries have therefore been established in USA and Denmark to help understand JORRP population's characteristics. The purpose of this study was to describe our pediatric serie of JORRP and to start a local registry.

Methods

Every patient diagnosed and treated for JORRP in the Head and Neck Pediatric Department of two tertiary-care hospitals was included in this study. 43 pediatric patients treated between 1980 and 2017 were thus included. Data regarding demographics, patients' history, surgery, Derkay&Wiatrak score, HPV genotype, histology, and outcome were collected retrospectively in spring 2017.

Results

In our serie, 23 patients were female and 20 were male. The mean age at diagnosis was 37,5 months-old (1-108 months). The average number of surgical procedures underwent by patient's during their follow up in the pediatric centers (up to 18 years-old) was 9,3. Seven cases of dysplasia (16,2%) were reported in this serie (6 low-grade dysplasia, 1 high-grade dysplasia). 5 patients presented a tracheal extent of the disease and 3 patients had pulmonary lesions. A safety tracheotomy had to be performed in one patient. Surgical after effects occurred in 7 patients.

Conclusion

In our experience, the JORRP was associated with a long-term treatment and follow-up including multiple surgeries. 16,2% of patients presented dysplasia. Further descriptive series, immunotherapy essays and therapeutic vaccination impact studies should lead to a better knowledge of this uncommon disease and how to treat it.
HN 06-08
PROSPECTIVE AND RETROSPECTIVE MONITORING FOR JUVENILE ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS (JORRP) IN THE UNITED STATES

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Background / Objectives

Juvenile onset recurrent respiratory papillomatosis (JORRP) is a rare but serious disease characterized by recurrent growth of wart-like lesions in the respiratory tract. Papillomas are presumably caused by vertical transmission of human papillomavirus (HPV) types 6 or 11, types which also cause genital warts. In the United States, both quadrivalent and 9-valent HPV vaccine, introduced in 2006 and 2015, respectively, protect against infection with these HPV types 6 and 11. HPV vaccination is routinely recommended for U.S. adolescents at age 11 or 12 years, with catch-up vaccination recommended for females through age 26 years. We aimed to establish a national registry for monitoring JORRP burden of disease in the HPV vaccine era.

Methods

Since January 2015, a prospective, multicenter study has been enrolling patients aged <18 years with JORRP presenting for care at participating U.S. pediatric otolaryngology clinics. Clinical disease history is abstracted from medical records, patient demographics and maternal characteristics are reported by mothers, and tissue and brush biopsy from papilloma specimens are tested for 37 types of HPV DNA using Linear Array. We calculated descriptive statistics including proportions, medians, and interquartile ranges (IQRs) using SAS 9.4.

Results

Through December 2016, 101 prevalent cases of JORRP were reported from 15 participating clinics. Among the 101 case-patients, median age at diagnosis was 3 years (IQR: 2–6 years) and median age at enrollment was 8 years (IQR: 5–12 years). In total, 56 (55.4%) were non-Hispanic white, 20 (19.8%) were non-Hispanic black, and 25 (24.8%) were another or unknown race/ethnicity. About half were male (57, 56.4%). Nearly all (93, 92.1%) had been delivered vaginally, and the majority (64, 63.4%) were first-born children. Among 101 mothers of case-patients, median maternal age at delivery was 21 years (IQR: 19–26 years). Few (10, 9.9%) had a
known history of genital warts, and none reported receiving any HPV vaccine before delivery. Among 21 case-patients with available HPV typing results, any HPV was detected in 20 (95.2%); HPV type 6 was detected in 15 (71.4%); and HPV type 11 was detected in 4 (19.0%). None were positive for >1 type of HPV.

Conclusion

JORRP case-patients were commonly first-born children delivered vaginally by unvaccinated young mothers. HPV types 6 or 11 were detected in nearly all case-patients tested, even though most mothers did not report a known history of genital warts. Increasing HPV vaccine uptake in the target age group could prevent or eliminate JORRP caused by vaccine-type HPV infections in the United States. Continued monitoring is ongoing.
Background / Objectives

Head and neck HPV infection, which includes epithelium from oral cavity, oropharynx, larynx, hypopharynx and sinonasal tract, represents a serious health care problem because of the association with cancer. About 90% of the head and neck cancers are squamous cell carcinoma (HNSCC). The aetiology of the HNSCC is changing, the tobacco and alcohol consumption are major, common risk factors, but now the role of HPV infection in the development of HNSCC is rising. The objective of this study was to analyze the HPV infection in three biopsies from larynx and one of the oral cavity with suspected papillomatosis.

Methods

The HPV detection were performed in the 4 fresh biopsies by the CLART®HPV2 (Genomic, Spain), this assay detected 35 genotypes (HPV -6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -61, -62, -66, -68, -70, -71, -72, -73, -81, -82, -83, -84, -85 and 89). To detect possible infections by other HPVs, all samples were submitted by conventional molecular analysis, using a MY09/11 primer’s and positive samples were sequenced.

Results

The histological result of the 4 patients (two males and two females, mean age 49.0 years) shows that the lesion are compatible with papillomatosis and in one cases was diagnosed with in situ carcinoma (CIS). The PCR confirmed the HPV infection in two samples, one with HPV 6 and the other with HPV 34 both from the larynx. The HPV 34 was detected in the sample with the CIS diagnosis.

Conclusion

The detection of the HPV 6 was the expected because, as the literature refers, the strong association of laryngeal papillomatosis to the low risk genotypes, namely HPV 6 and HPV 11. The detection of the probable high risk genotype HPV 34 in CIS cases is very curious since there is no description in the literature of this genotype in cases laryngeal papillomatosis infections neither in HNSCC cases.
QUALITY OF LIFE IN SURVIVORS OF OROPHARYNGEAL CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS OF 1366 PATIENTS

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Background / Objectives

Human papillomavirus (HPV)-associated oropharyngeal cancer (OPC) is rapidly increasing in incidence and has a favourable prognosis compared to HPV-negative disease. Current combined therapies include significant risks of morbidity for the growing group of survivors. This systematic review and meta-analysis investigates how treatment affects quality of life (QoL) in survivors of oropharyngeal cancer.

Methods

PubMed, EMBASE, and the Cochrane Library were systematically searched for all studies reporting patient-assessed QoL at least one year after treatment for OPC. In a meta-analysis, weighted average QoL scores from the four most commonly utilised QoL instruments were compared to baseline and reference group scores using the concept of minimal clinically important difference. The meta-analysis included data from 1366 patients from 25 studies and 12 countries.

Results

The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 (EORTC QLQ-C30) was answered by 704 patients, 644 patients answered the EORTC QLQ Head and Neck-35 (H&N-35), 474 patients answered the University of Washington Quality of Life Questionnaire (UWQOL), and 381 patients answered the M. D. Anderson Dysphagia Inventory (MDADI). Moderate to large clinically important deteriorations in QoL were found in the domains dry mouth and sticky saliva for the EORTC QLQ-H&N35, in the domains saliva, chewing, swallowing, speech, taste, appearance and shoulder for the UW-QOL, and in the global, physical, and emotional subscales for the MDADI.

Conclusion

In conclusion, survivors of OPC face clinically important deteriorations in QoL that most markedly centre on xerostomia, dysphagia and chewing. These ailments indicate a potential for improvement in patient management.
HN 07-02
MAPPING THE IMMUNE SUPPRESSIVE MICROENVIRONMENT IN SENTINEL LYMPH NODES DRAINING HPV-NEGATIVE HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Background / Objectives

Tumor-mediated immune suppression of the sentinel lymph node (SLN) can hamper the development of an efficient anti-tumor immune response. Expression of inhibitory molecules on dendritic cell (DC) subsets that have migrated from the tumor region or that reside in these SLN, can negatively influence T cell priming and activation.

Methods

In two independent cohorts of tumor-negative SLN draining HPV-negative oral cavity squamous cell carcinomas, (n=9 USA and n=12 NL), we performed multi-parameter flow cytometry to map the immune suppressive microenvironment.

Results

In both cohorts we observed that migratory Langerhans cells (LC) and interstitial DC (iDC) had an activated phenotype with high expression of co-stimulatory molecules, but this coincided with high expression of the immune checkpoint molecule PD-L1. In contrast, lymph node resident (LNR) DC subsets had an immature phenotype, scarce expression of PD-L1, but abundantly expressed the inhibitory molecule immunoglobulin-like transcript 4 (ILT4). ILT4 on tolerogenic dendritic cells (DC) has been described to result in induction of regulatory T cells (Treg). Indeed, increased expression of ILT4 (mean fluorescence index) on CD14- LNR-DC was found to correlate with increased frequencies of activated Treg (aTreg) in these SLN (Pearson r= 0.84, p<0.001). The same was true for expression of its ligand, HLA-G, on this subset (Pearson r=0.79, p<0.01).

Conclusion
Our data suggest that different DC subsets present in SLN draining HPV-negative HNSCC, may use different suppressive pathways to impair the generation of an effective anti-tumor immune response. It might thus take interference of both the PD-L1/PD-1 and the ILT-4/HLA-G pathways to effectively lift immune suppression and improve anti-tumor reactivity.
INTRATUMORAL HPV IMMUNINITY AS PREDICTOR OF RESPONSE TO THERAPY

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Background / Objectives

HPV-associated oropharyngeal squamous cell cancer (OPSCC) is a distinct clinical entity with a much better prognosis after (chemo)radiotherapy than HPV-negative OPSCC, a feature that has been linked to the local presence of a more dense immune infiltrate. To evaluate the relation between clinical outcome, immune cell infiltration and the possibility of the immune system to react against potent virally-derived antigens, we performed an in-depth analysis of freshly isolated tumor-infiltrating lymphocytes from a cohort of 97 HPV16-positive and -negative OPSCC patients.

Methods

Fresh tumor tissue was processed to obtain tumor infiltrated lymphocytes (TILs). Furthermore, tumor tissue was archived and subsequently used for HPV DNA typing, test the expression of p16INK4a and quantification of T-cell infiltration. TILs were phenotypically analyzed and their capacity to expand and produce cytokines following stimulation with HPV16 E6 and E7 antigens or phytohemagglutinin (PHA) was tested using proliferation assays and flow cytometry. Findings were validated in an additional cohort of 75 HPV+ OPSCCs from the publicly available cancer genomic atlas (TCGA) database. The direct growth inhibiting effects of the TIL-produced cytokines was tested on five different OPSCC cell lines, either with or without cisplatin, the commonly used chemotherapeutic agent.

Results

Patients with a HPV+ OPSCC have a better overall survival (OS) than HPV-negative OPSCC patients. Within these HPV+ OPSCC patients the majority displayed a HPV-specific T-cell response, predominated by CD4+ T cells. HPV-immunity was strongly correlated to a more favorable clinical outcome. The HPV-reactive T cells produced IFNg, TNFa and IL-17, thus, exhibiting a T-helper type 1 (Th1)/Th17 profile. A high expression of CD4 and IFNg also correlated with better overall survival in the TCGA dataset. Notably, the OPSCC of patients displaying an intratumoral HPV16-specific T cell response were significantly higher infiltrated by T cells expressing the with IFNg-
production associated transcription factor Tbet than the other patients. Culture of OPSCC tumor cell lines in the presence of IFNg and TNFa lowered their proliferation, while the combination with cisplatin resulted in increased apoptosis of the tumor cells.

**Conclusion**

In conclusion, the presence of Th1-oriented HPV16-specific T cells in pre-treated HPV+ OPSCC better controls tumor growth (lower T and N stage), makes the tumor more susceptible for cisplatin and results in better OS of the patients. Therefore, these HPV16-specific TILs can be used as predictor of clinical outcome upon therapy.
HN 07-04
HPV THERAPEUTIC VACCINE FOR HEAD AND NECK CANCER: ROLE OF RESIDENT MEMORY T CELLS


PARCC INSERM U970 (France)

Background / Objectives

Tissue-resident memory T cells (Trm) represent a new subset of long-lived memory T cells that remain in tissue and do not recirculate. Although they are considered as early immune effectors in infectious diseases, their role in cancer immunosurveillance remains unknown.

Methods

In a preclinical model of head and neck cancer, we show that intranasal vaccination with a mucosal vector, the B subunit of Shiga toxin, induces local Trm and inhibits tumour growth (1). As Trm do not recirculate, we demonstrate their crucial role in the efficacy of cancer vaccine with parabiosis experiments (2). Blockade of TFGβ decreases the induction of Trm after mucosal vaccine immunization, resulting in the lower efficacy of cancer vaccine. In contrast, using fingolimod (FTY720) which blocked the recruitment of circulating effector cells, we demonstrate the major role of Trm over effector T cells to control tumor growth following cancer vaccine administration. In order to extrapolate this role of Trm in humans, we show that the number of Trm correlates with a better overall survival in lung cancer in multivariate analysis.

Conclusion

The induction of Trm may represent a new surrogate biomarker for the efficacy of a therapeutic HPV cancer vaccine. This study also argues for the development of vaccine strategies designed to elicit them.

References


Well powered HPV serology sub analyses in the Head and Neck 5000 study

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Background / Objectives

Human papillomavirus (HPV) 16 seropositivity is associated with survival in people with oropharyngeal cancer. The role of other HPV serotypes and HPV at other head and neck cancer sites is unclear. We used data from Head and Neck 5000 to estimate the prevalence of HPV antibodies in people with head and neck cancer at different sites and to describe differences in survival.

Methods

We used data from a large prospective UK clinical cohort of people with a new diagnosis of head and neck cancer recruited from April 2011 to December 2014. Prior to treatment, participants completed questionnaires and provided a blood sample. Clinical details were extracted from clinical notes. Review of pathology reports is ongoing so figures reported in the abstract may be subject to change. Blood samples were analysed using multiplex serology; HPV16 E6 seropositivity was defined as >1000 median fluorescence intensity. The role of other HPV16 antigens and antigens from other serotypes was explored. Date of death was obtained through record linkage with a mean follow up time of 2.6 years. Cox regression models included age, gender, stage (not for primary of unknown origin, PUO), treatment intent, smoking and co-morbidity.

Results

The prevalence of HPV 16E6 positivity was 68.5% in 1,606 people with oropharyngeal cancer and increased to 71.8% when people with a response to at least three HPV16 or HPV18 antigens or paired E6 and E7 of other high HPV types were added. The survival of these additional people with oropharyngeal cancer defined as HPV positive was similar to those based on HPV16 E6 serology alone so they are included in survival analyses. The prevalence of HPV positivity was 55.9% in 134 people with PUO; 13.5% in 104 people with nasopharyngeal cancer; 9.8% in 195 people with hypopharyngeal cancer; 5.7% in 53 people with nasal cavity cancer and only 2.4% in 1120 people with oral cavity cancer; 2.1% in 911 people with laryngeal cancer and 1.8% in 115 people with major salivary gland cancer. Adjusted hazard ratios were 0.34 (95% CI 0.23 to 0.51) for oropharyngeal cancer; 0.17 (95% CI 0.02 to 1.45) for PUO; 0.25 (95% CI 0.07 to 0.90) for nasopharyngeal, hypopharyngeal and nasal cavity cancer combined and 0.85 (95% CI 0.39 to 1.83) for oral cavity, laryngeal and salivary gland cancer combined.
Conclusion

HPV seropositivity in the UK is high in people with oropharyngeal cancer and PUO. People with a PUO who are HPV seropositive appear to have a similar survival to people with oropharyngeal cancers. HPV seropositivity is lower in other sites and may be related to survival for nasopharyngeal, hypopharyngeal and nasal cavity cancer but not for oral cavity, larynx and salivary gland cancer.
HN 08-05
USING HPV SEROLOGY FOR PREDICTING RECURRENCE – SUMMARY OF AVAILABLE DATA

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Background / Objectives

Not all patients with human papillomavirus-driven oropharyngeal cancer (HPV-OPC) have favorable outcomes. Accurate risk stratification has important implications for treatment and post-treatment surveillance. Yet, there are few clinical and no molecular markers of HPV-OPC recurrence. Several small studies (range: 52-115 patients) have evaluated HPV16 E6 antibodies as a potential prognostic marker; however, results are conflicting. Five prior studies evaluated pre-treatment HPV16 E6 antibodies and risk of recurrence among HPV-OPC patients; 1 study reported that increased pre-treatment HPV16 E6 antibody levels were associated with an increased risk of recurrence, 2 studies reported that HPV16 E6 seropositivity was associated with a reduced risk of recurrence and 2 studies found no associations. Six studies assessed the association between change in HPV16 E6 antibody levels post-treatment and risk of recurrence. Five out of 6 studies reported HPV16 E6 antibody levels decreased post-treatment; only 1 study reported an association between stable post-treatment HPV16 E6 antibody levels and risk of recurrence. The specific details of each study as well as future directions will be discussed.

Methods

N/A

Results

N/A

Conclusion

N/A
Considerations in prevention of HPV-driven oropharyngeal cancer

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Background / Objectives

Human papillomavirus (HPV) is an important cause of oropharyngeal cancer (OPC) in many world regions, the large majority of HPV-driven OPC being caused by HPV type 16. In countries with high human development index, particularly in the United States (US) and Europe, both the incidence of OPC and the attributable proportion due to HPV are rising. In the US, the burden of OPC is now greater than cervical cancer, given the success achieved by cervical cancer screening. This increasing OPC disease burden highlights the need for parallel preventive measures like screening. Yet, there have been arguments against additional research on HPV-driven OPC screening, including, the promise of primary prevention through HPV vaccination, the currently low incidence rates, and favorable outcomes with current therapy compared to non-HPV driven head and neck cancers. This talk aims to discuss issues related to screening for HPV-driven OPC and highlight areas which require additional future research in the following subject areas: disease incidence, possible screening biomarkers, diagnostics among screen positives, and treatment.

Methods

Results

Conclusion

References
Modeling the impact of sex-neutral vaccination

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Background / Objectives

Vaccinating boys may be an appealing complementary strategy for the prevention of HPV-related diseases and has been introduced in a few countries already. It is important to understand the impact of vaccinating boys on tumor-site specific cancers and pre-cancers. A key parameter when assessing the impact is the herd immunity effect from a girls’ only program.

Methods

We developed a Bayesian synthesis framework to account for all vaccine type-related cancers and herd immunity effects from vaccinating girls and boys. The model has been calibrated to six countries from different regions in Europe. For this presentation, we evaluated the impact of vaccinating boys on oropharyngeal cancers in males and females.

Results

Vaccinating 40% of boys in six European countries prevents 687 cancer cases per 100,000 women and 445 cancer cases per 100,000 men. Of those, 278 cancer cases per 100,000 individuals are prevented in the oropharyngeal region, with 95% of the gain attributed to men. Vaccinating 40% of boys prevents 25% of all HPV-related oropharyngeal cancers.

Conclusion

Vaccinating boys is expected to contribute substantially to a decrease in the occurrence of oropharyngeal cancers.
Occupational exposure to HPV: How can we best protect ourselves

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Background / Objectives
Occupational exposure to HPV is a potential risk factor for development of HPV-related diseases among surgeons of many specialties.

Methods
A prospective vaccination program of house staff and attending surgeons in Otolaryngology, Urology, General Surgery and Obstetrics/Gynecology has been developed. Pre- and post-vaccination HPV titers will help determine the immunogenicity of vaccination in this occupationally-vulnerable, previously un-vaccinated high risk group.

Results
Preliminary data from this prospective study, along with the rationale and potential application to surgeons and other health care workers will be presented.

Conclusion
Health care workers may be at risk for contracting HPV-related disease of the aero-digestive tract through occupational exposures. Surgeons in the fields of Otolaryngology, Urology, General Surgery and OB/Gyn may be particularly vulnerable. Introduction of vaccination programs targeting 11-12 year old boys and girls have left these surgeons unvaccinated. If found to be immunogenic, administration of the HPV-9 vaccine in adult male and females surgeons may protect them from contracting disease.
AN EVALUATION OF RRP RISK FACTORS: IS IT AGE OF DIAGNOSIS OR HPV TYPE?

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Background / Objectives

Recurrent respiratory papillomatosis 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 but only account for incidence. The disease course that each person follows is variable. Some run an indolent course while those who either 1) undergo 10 or more procedures for RRP or 2) undergo 4 or more surgeries within a 12 month period or 3) develop disease distal to the larynx or 4) undergo a tracheotomy are considered to have aggressive disease. Ever since an association between aggressive recurrent respiratory papillomatosis and HPV 11 was discovered, clinicians have wanted to obtain HPV typing as part of their workup. What information would HPV typing provide and how much of the variability could be predicted if typing was known?

Methods

Together, we will review past data sets showing that young age of onset, HPV 11 and aggressive course are all associated. Then we will use multiple regression and Fast and Frugal Trees to determine the utility of HPV type to predict aggressiveness.

Results

From our largest data set presented at HPV 2017 in Cape Town, 338 subjects with ≥ 1 year follow up and with a single HPV type were classified.

<table>
<thead>
<tr>
<th>Clinical Course HPV type</th>
<th>indolent</th>
<th>aggressive</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 6</td>
<td>47 (27%)</td>
<td>124 (73%)</td>
<td>171 (100%)</td>
</tr>
<tr>
<td>HPV 11</td>
<td>16 (15%)</td>
<td>90 (85%)</td>
<td>106 (100%)</td>
</tr>
</tbody>
</table>

In multiple logistic regression, aggressiveness was associated with JoRRP but not with HPV type.
Conclusion

In multiple analyses we observe that young age of disease diagnosis is far more closely associated with aggressiveness than is HPV type.
Current status of HPV-vaccination in The Netherlands

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Background / Objectives

Many (young) sexually active Dutch women and men become infected with HPV. Since 2010, 12-year-old girls are vaccinated with the bivalent vaccine through the National Immunisation Programme (NIP) to prevent HPV16- and HPV18-related cervical cancer. A catch up programme was implemented for girls aged 13-16 years in 2009. Implementation of routine HPV-vaccination in the NIP in The Netherlands is accompanied with monitoring of vaccine effectiveness and side effects in a population-based setting.

Methods

Monitoring to obtain insight into (future) effects of HPV-vaccination include: Monitoring of vaccine coverage using individual based registration of vaccinations (Praeventis database); Safety of vaccination through enhanced passive surveillance of adverse events through the National Pharmacovigilance Center Lareb as well as specific studies in response to adverse events signals; Cohort studies among young women eligible for HPV-vaccination to obtain insight into immunogenicity and vaccine-effectiveness against type-specific HPV infection; and a repeated cross-sectional survey on HPV prevalence in STI-clinic visitors. Mathematical modelling is performed on long term impact on disease burden and (cost)effectiveness.

Results

Vaccine uptake increased from 52% (2009) to 61% (2014-2015), but preliminary data suggest a decrease in 2016. Local reactions and systemic adverse events are commonly reported after HPV vaccination, but are mostly mild and transient. As of yet, there is no evidence of a statistically significant association with serious adverse events. Vaccine effectiveness estimates for 12-month persistent high-risk (hr) HPV-infections among young females hrHPV-negative at time of HPV-vaccination was above 95% for vaccine types 16/18. Among female STI-visitors vaccine-effectiveness against prevalent HPV16/18 infection was 90%. Both studies showed indications for cross-protection.

Conclusion

Monitoring of HPV-vaccination programme shows high vaccine-effectiveness against (persistent) HPV16/18-infections. No evidence was found for serious adverse events associated with vaccination. The current programme for girls is expected to lead in the future to annually 350 fewer women with cervical cancer and 100 fewer female deaths due to this cancer. Efforts to increase vaccination coverage need to be emphasized. In the past years, the occurrence of HPV-related cancers in males has steadily increased. Recent findings show the vaccine protects also against penile,
anal, vaginal and vulvar cancer. There are indications for the prevention of oropharyngeal cancers. In response to these novel insights, the Ministry of Health asked the Health Council to prepare an update of their advice in 2008.

References

*on behalf of the HPV-research team RIVM: Birgit van Benthem, Hans Bogaards, Robine Donken, Jeanet Kemmeren, Audrey King, Fiona van der Klis, Birthe Lehmann, Scott McDonald, Hester de Melker, Liesbeth Mollema, Hella Pasmans, Venetia Qendri, Marianne van der Sande, Tessa Schurink, Hans van Vliet, Pascal van der Weele, Petra Woestenberg
Sekse-neutrale’ vaccinatie: zin of onzin (Session: Huidige situatie en ontwikkelingen m.b.t. prophylactische vaccinatie)

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Background / Objectives

De opkomst bij het huidige meisjesvaccinatieprogramma is ongeveer 60 procent en ligt lager dan de vaccinatiegraad van andere vaccins in het rijksvaccinatieprogramma. Het vaccineren van jongens kan een interessante strategie zijn, omdat de aanbestedingsprocedure de prijs van het vaccin heeft gedrukt en het aantal doses is verminderd van drie naar twee. In plaats van het vaccineren van jongens zou ook overwogen kunnen worden om de opkomst bij meisjes te verhogen. Om een gefundeerd advies te kunnen geven of jongensvaccinatie zinvol is, is het belangrijk om de kosteneffectiviteit van jongensvaccinatie te berekenen.

Methods

We hebben de kosteneffectiviteit berekend van jongensvaccinatie door epidemiologische analyses te combineren met een Bayesiaans model. Dit model wordt gebruikt om de indirecte effecten van meisjes- en jongensvaccinatie schatten die optreden ten gevolge van de zogenaamde kudde-immuniteit. Voor de kosteneffectiviteitsberekeningen zijn de richtlijnen van het NZA gevolgd.

Results

Het vaccineren van 40% van de jongens naast de huidige 60% van meisjes levert dezelfde winst op in aantal levensjaren als het verhogen van de opkomst bij meisjes van 60 naar 80%. Jongensvaccinatie is kosteneffectief onder het laatst gepubliceerde HPV vaccintarief. Jongensvaccinatie blijft kosteneffectief als de opkomst bij meisjes stijgt van 60 naar 80%.

Conclusion

Het vaccineren van 2 jongens levert ongeveer evenveel gezondheidswinst op als het vaccineren van 1 extra meisje. Niettemin is het vaccineren van jongens zeer effectief bij het huidige tarief van het vaccin en de opkomst in Nederland.
Background / Objectives


Methods


In het uitvoeringskader zijn de aspecten van het bevolkingsonderzoek in meer detail uitgewerkt. Alle partijen werken conform het uitvoeringskader. Deze wordt jaarlijks geactualiseerd en is ook op de website te vinden.

Results

Deelnemende vrouwen laten nog steeds een uitstrijkje maken in de huisartsenpraktijk. Dit uitstrijkje wordt eerst beoordeeld op de aanwezigheid van hrHPV. Als er afwijkende cellen zijn, vindt door de huisarts verwijzing naar de gynaecoloog plaats. Als er geen afwijkende cellen zijn, krijgt de vrouw het advies om na 6 maanden een nieuw uitstrijkje te laten maken bij de huisartsenpraktijk. Dit vervolguitstrijkje is ook onderdeel van het bevolkingsonderzoek. Vrouwen die hrHPV positief zijn, ontvangen bij de uitslagbrief ook een uitslagfolder. Non-responders krijgen na 4 maanden een herinneringsbrief en de mogelijkheid om een zelfafnameset aan te vragen. Deze is geschikt voor het testen op hrHPV.

Vijf screeningslaboratoria zijn gecontracteerd voor de uitvoering van de hrHPV-test en de cytologie van het bevolkingsonderzoek. Vanuit kwaliteit van de cytologie, kosten, toekomstbestendigheid en aansturing is het aantal screeningslaboratoria beperkt ten opzichte van het oude bevolkingsonderzoek.

Conclusion
Het nieuwe bevolkingsonderzoek is succesvol ingevoerd. Door de inrichting kunnen verbeteringen en innovaties snel doorgevoerd worden. Er vindt intensieve monitoring plaats om eventuele overbehandeling snel te signaleren zodat bijsturing mogelijk is.
Human Papillomavirus (HPV) virion induced cancer and subfertility

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Background / Objectives

In the natural history of HPV infections, HPV virions can induce two different pathways, namely the infectious virion producing pathway and the clonal transforming pathway. An overview is given of the burden that is associated with HPV infections that both lead to cervical cancer and temporal subfertility. That HPV infections cause serious global health burden due to HPV-associated cancers is common knowledge, but that it is also responsible for a substantial part of idiopathic subfertility is greatly underestimated. The bulk of the detected HPV DNA whether in men or women is however infectious from origin. Because the dissociation between HPV viruses and HPV virions or infection and disease remains difficult for clinicians as well as for HPV detection, we propose a review of the different effects caused by the two different HPV virion induced pathways, and highlight the mechanisms that are responsible for causing transient subfertility and cancer.

Methods

When reviewing the evidence it seems evident that determining the origin of the detected HPV DNA is the key to predict the impact of the HPV infection.

Conclusion

When the detected viral HPV DNA originates from a dividing cell, the detected HPV DNA is never infectious (dividing cells do not support virion production) and does not affect fertility, but the viral DNA can transform the dividing cell it resides in, which could in time lead to pre-cancer and cancer. High-risk HPV oncogenic proteins can transform dividing cells or lead to blocking of the cell division of the early embryo. It always leads to a self-limiting HPV infection since no progeny virions can be made and the HPV life cycle ends.

Viral HPV DNA originating from non-dividing differentiated cells or from free virions, is infectious and exerts its deleterious effects thru weakening or incapacitating the cells it resides in. For the already dying epithelial cells the new virions are released when the cells desquamate and allow to restart the HPV life cycle. In embryos the accumulation of newly formed virions in the syncytiotrophoblasts results in the weakening of the implantation bond with the endometrium and diminished energy uptake leads to miscarriage. For spermatozoa the binding or internalization of viral DNA also leads to a decrease in functionality of sperm.
Independent of the number of HPV types detected, serial measurement of type specific viral load allows to identify the origin of the detected HPV DNA and makes it possible to assess the impact of the HPV infection for cancer screening or fertility.

Although HPV is not a lytic virus, harboring its DNA will ultimately lead to cell death or immortality depending on the cell type.

References

CURRENT HPV RECOMMENDATIONS AND VACCINATION COVERAGE IN BELGIUM – REASONS TO WORRY OR TO REJOICE?

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Background / Objectives

Recommendations regarding HPV vaccination for young adolescent woman have been in place since 2007 in Belgium, and since 2010, HPV vaccines have been offered free-of-charge through a school-based system to all girls in the 1st or 2nd year of secondary school in Belgium. The recommendations were thoroughly discussed and updated in 2017, and led to specific recommendations for boys, MSM, and immunocompromised patients. The opportunity was taken also to update information on safety and long term data for the bivalent, quadrivalent and nonavalent HPV vaccine as well as data on HPV vaccination in women 27 to 45 years of age and women with pre-existing HPV infection.

In 2012-2013 vaccination coverage in the recommended free-of-charge age group was assessed in Flanders and the Walloon region of Belgium. The coverage for the 3rd dose of HPV vaccination was 29% in the Walloon region, 36% for Capital Region of Brussels and 83% in the Flemish region.

In 2016, the HPV vaccination coverage was measured again in girls (born in 2000) living in Flanders who were eligible for a free of charge 3-dose HPV vaccination scheme four years before.

Methods

The 2016 survey was done using WHO’s Expanded Program on Immunization two-stage cluster sampling technique. Parents of 488 adolescent girls were interviewed at home and vaccination documents were copied after consent was given. Apart from vaccination status and socio-demographic data, parents were also asked to complete a general questionnaire on vaccine confidence/hesitancy. Vaccination data were checked against the electronic Flemish vaccination registry (Vaccinnet) and still missing data, were retrieved from the GP, pediatrician and School Health Service.

Results

Vaccination coverage (95%CI) for the 1st, 2nd and 3rd dose was respectively 92.3% (89.7-94.8%), 92.2% (89.6-94.8%) and 89.5% (86.5-92.4%). When classified according to the current 2- or previous 3-dose recommended schedule, the coverage
for being correctly vaccinated increased to 91%. Of these vaccines, 89% were administered through the school health system. Factors associated with incomplete or non-vaccination were living in a larger city, having a lower family income and having a mother or a father of non-Belgian origin. Confidence in vaccination was high.

Conclusion

The HPV vaccination program of girls seems consolidated in Flanders but not in the other Belgian Regions. However, care should be taken to maintain the confidence in this vaccine in Flanders as different stories on alleged side effects are currently circulating on social media.

References


Kan de triage van HPV positieve vrouwen verbeterd worden

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Abstract for local workshop.

Met de invoering van de HPV test als primaire screening test voor het bevolkingsonderzoek (BVO) baarmoederhalskanker (BMHK) is de gevoeligheid voor het opsporen van (pre)kanker aanzienlijk toegenomen waardoor de kans op fout negatieve uitslagen sterk verminderd is. Ondanks het opstellen van richtlijnen voor klinische validatie van HPV testen, waardoor het aantal gedetecteerde transiente HPV infecties sterk is verminderd, kan een klinisch gevalideerde HPV test onvoldoende verschil maken tussen een voorbijgaande irrelevante HPV infectie en een persisterende HPV infectie die geassocieerd is met (pre)kanker. Om overbelasting te voorkomen van het medisch circuit dat verplicht is alle vrouwen met een positieve klinisch gevalideerde HPV test verder te evalueren moet een additionele test, een zogenaamde triage test, op cervixmateriaal van HPV positieve vrouwen uitgevoerd worden. Best geclassificeerde triage testen zijn cytologie, HPV 16/18 typering of een combinatie daarvan. Ook dubbelkleuring van uitstrijkjes met p16INK4a/ Ki-67 of moleculaire bepaling van de methylation levels van de promotor regio’s van genen betrokken bij het ontstaan van BMHK met name FAM19A4 en miR 124-2 zijn goede triage markers. In het nieuw opgestarte Nederlandse BVO is momenteel gekozen om HP positieve vrouwen te trieren mbv reflex cytologie op het cervix materiaal, waarop de HPV test verricht is en indien het uitstrijkje geen celafwijkingen aantoont, dit cytologisch onderzoek te herhalen na 6mnd. In de voordracht zullen de voor- en nadelen van de verschillende triage testen worden besproken alsmede ook de ontwikkelingen die zich op dit terrein voordoen en die kunnen leiden tot een volledig moleculaire screening op door vrouwen zelf-afgenomen cervix/vaginaal materiaal.

Methods

For local Workshop

Conclusion

For local workshop
Background / Objectives

Herd protection against HPV is governed by the probability of infection transmission, the duration of the infection, and sexual activity pattern, which varies in different populations. As a result, overall effectiveness of HPV vaccination at a population level, that is the sum of vaccine efficacy and herd protection, is population specific and, within the same population, type specific. The heterogeneity of herd protection affects the vaccination program effectiveness in different populations and against different HPV types. We use the IARC transmission-dynamic model to illustrate the herd immunity effect of HPV vaccination against different HPV types and in populations with different HPV pre-vaccination prevalence.

Methods

We simulated A) HPV16 and HPV45 infections within the same population. We compared the impact of vaccination on type-specific prevalence and on cervical cancer prevention. For HPV45, we also assessed the effect of cross-protection from HPV16/18 vaccines. B) HPV16 in populations with different background pre-vaccination prevalence (range 1% to 8%). We compared the impact of vaccination on population-specific prevalence and calculated the coverage adequate to meet selected HPV control thresholds.

Results

Prevalence reduction (PR) attributable to vaccination was larger for HPV45 than for HPV16, regardless coverage levels. The difference was wholly attributable to herd immunity. With 70% and 50% (or higher) coverage, assuming girls-only and gender-neutral vaccination respectively, the incidence of HPV16-related cancers decreased from 4.1 to <1 per 105 women, whereas HPV45-related cancers were virtually eliminated. Similar estimates were obtained when levels of cross-protection against HPV45 were assumed to be 50% (or higher) and vaccination coverage at least 70%. For any given coverage, HPV16 PR attributable to vaccination was larger in populations with lower pre-vaccination prevalence. This finding was consistent across populations with different sexual activity patterns.

Conclusion
Due to herd immunity, HPV16 is more difficult to control and eliminate than other less frequent/persistent types. Partial cross-protection may be sufficient to eliminate cervical cancer associated with HPV45 and, possibly other HR HPV types that may share with HPV45 lower duration of infectious period and less ability to produce cancer than HPV16 and 18. Furthermore, HPV16 is more difficult to control and eliminate from populations with higher pre-vaccination prevalence and minimal coverage thresholds for HPV control or elimination depend on the pre-vaccination prevalence and sexual activity patterns of each population.
Sex-neutral vaccination: the role of tender pricing

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Background / Objectives

In many countries, vaccination uptake among girls remains below the target level. Vaccinating boys may be an appealing complementary strategy for the prevention of HPV-related diseases, especially since tender negotiations and reduced dosing schemes have driven down the cost of vaccination.

Methods

We examined HPV vaccine tender-based prices published in Europe since 2007. On the basis of the latest available HPV vaccine prices, we evaluated whether extending boys is a cost-effective addition to girls’ only vaccination after adjusting for herd immunity effects. We will present health effects and economic benefits of sex-neutral vaccination in countries from different regions in Europe.

Results

HPV vaccine tender-based prices have dropped by 60% to 80% of the ex-factory prices since 2007, the first year of implementation in tender-based settings. This has led to a substantial decrease in incremental cost-effectiveness ratios (ICERs) of girls-only vaccination compared to no vaccination; the ICERs ranged from €500 (95% CrI: 0 - 1,000) per life-year gained in Latvia to €5,000 (95% CrI: 4,000 - 6,000) per life-year gained in Austria. The ICERs of adding boys to a girls-only program showed a larger variation among countries than the ICERs of girls-only versus no vaccination, but in all countries the ICERs remained below the country-specific thresholds for a cost-effective intervention.

Conclusion

Vaccinating boys is only modestly less efficient than increasing uptake among girls. Sex-neutral vaccination is likely to be cost-effective under tender-based vaccine prices.
Gender-neutral vaccination program: real life example

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Background / Objectives

Austria was the first country to recommend gender neutral HPV vaccination in 2007. Countries like the US and Australia have moved forward to a gender neutral HPV vaccination, since 2014 Austria has a school based program where girls and boys from 9-11 get the HPV vaccine for free.

Methods

The current status of the Austrian HPV vaccination program is reported.

Conclusion

The target population is 9-10, the school based program has achieved an uptake for girls and boys of >60%. The 2-dose schedule is used. The early age of vaccination has facilitated the program. A catchup for 12-14 year old adolescent is in place, the receive the 2 doses at a reduced prize of 50€/dose. Since September 2016 the ninevalent vaccine is used in the public program, the transition went smoothly and is almost finished. Gender neutral vaccination is considered as cost-effective in Austria.

References


MSS 02-01
CONTRIBUTION OF CYTOLOGY

G. Ronco

Città della salute e della scienza di Torino (Italy)

Background / Objectives

Estimating the risk of developing CIN3 in HPV negative and in cytology and HPV double-negative women

Methods

The data of 4 randomised controlled trials (RCTs) comparing HPV-based to cytology-based cervical screened were pooled. All RCTs applied co-testing except NTCC phase 2. Women enrolled in such phase were excluded. In the cytology arm the cumulative incidence of CIN3 was computed for women with normal cytology at baseline. In the HPV arm the cumulative incidence of ICC was computed for women testing HPV negative at baseline and for women testing negative for both HPV and cytology at baseline.

Results

Some 115756 women were included in the analysis. Among them 53348 were from the control arm and were cytology-negative at baseline while 62408 were from the experimental arm and were HPV negative at baseline. Of the latter 59065 were double negative to HPV and cytology, while 3343 had abnormal cytology. The cumulative incidence of CIN3 within 5.5 years from baseline was 241.5 per 105 py (95%CI 202.9-287.3) in the cytology arm when considering cytology-negative women, 115.0 (91.1-145.1) in the experimental arm when considering all HPV-negative women and 85.8 (65.0-113.1) in the experimental arm when considering double negative women.

Conclusion

The difference in future risk of developing CIN3 between double-negative and HPV-only negative women is small compared to that between HPV-negative and cytology-negative women.
Background / Objectives

In human papillomavirus (HPV) based screening, several options are available for triaging HPV-positive women. The performance of a triage strategy can be measured by the positive predictive value for detection of underlying CIN3+ and the three to five year CIN3+ risk after a negative result.

Methods

We examined published screening studies with long-term follow-up and evaluated the performance of cytology, repeat HPV testing, and HPV16/18 genotyping. We also evaluated whether the performance of triage tests changes after multiple rounds of HPV-based screening.

Conclusion

Cytology and HPV16/18 genotyping have good performance in the first round after a program switch from cytology-based to HPV-based screening. Repeat HPV testing after 6 to 12 months among HPV positive women with normal cytology is associated with a low CIN3+ risk at the cost of a considerable number of extra colposcopy referrals. HPV-positive, triage-negative women have an elevated CIN3+ risk in comparison to HPV-negative women, suggesting that screening intervals should be determined separately for HPV-positive and HPV-negative women. After multiple rounds of HPV-based screening, risk differentiation by cytology remains strong but may be somewhat diminished for HPV 16/18 genotyping because of a larger proportion of incident infections.
MSS 02-05
Expected impact of 9-valent HPV vaccine (invited talk in MSS 02)

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Background / Objectives

A 9-valent HPV vaccine that provides broader protection against oncogenic HPV types than the 4-valent vaccine has now been licensed in the United States and Europe. However, it is not used in most countries in the world. We evaluated the cost-effectiveness of the 9-valent vaccine to inform recommendations by the World Health Organization.

Methods

We evaluated the contribution of the additional HPV types in the 9-valent vaccine (31/33/45/52/58) in cervical and other cancers. We then considered the proportion of these cancers that could be prevented by 2-valent, 4-valent and 9-valent vaccines (assuming 2-valent and 4-valent vaccines offer cross-protection), with and without herd effects. The incremental cost-effectiveness of the 9-valent vaccine compared to lower valency vaccines was then calculated.

Results

Most HPV-related cancers due to the additional types in the 9-valent vaccine are cervical cancers. The incremental benefit of the 9-valent vaccine compared to 2-valent and 4-valent vaccines differs depending on assumptions about cross-protection and herd effects. The relative cost-effectiveness of the 9-valent vaccine depends on the level of cross-protection expected from lower valency vaccines.

Conclusion

World Health Organization guidelines now include the 9-valent HPV vaccine as one of the recommended HPV vaccines alongside the 2-valent and 4-valent vaccines.

References

Background / Objectives

In Italy, the cohorts of women who were offered HPV vaccination in 2007/08 will reach the age for cervical cancer (CC) screening in 2017. The simultaneous shift from cytology-based screening to HPV test-based screening gives the opportunity for unprecedented reorganisation of CC prevention. Despite a wide consensus about the need of adopting different screening protocols for vaccinated women, no guidelines yet recommend different screening strategies (systematic review updated in 2015).

Methods

The ONS (National Screening Monitoring Centre) Directive and the GISCi (Italian Group for Cervical Screening) identified the consensus conference as the most suitable method for defining the research needs in order to define the best protocols in vaccinated women (1). The ONS and GISCi set up a promoting committee and a Jury. The promoting committee identified a panel of experts representative of Italian scientific societies involved in CC prevention, who defined the scope questions and commissioned several systematic review and modelling studies in order to answer the following questions:

Do the protocols for screening programs need to be changed upon the arrival of the cohorts of vaccinated women?

If so, which policy: tailored strategy or one size fits all strategy.

At what age should screening start?

With which test?

How often?

Should the strategy be different for the cohorts vaccinated in their 15th year (or later) with respect to those vaccinated in their 12th year?

Which actions need to be scheduled from now?
According to a systematic review on long term vaccine accuracy and the estimate of cumulative incidence of non-16/18 cancers in pre-screening era in Italy, the number of cancer non-vaccine-preventable cancers in unscreened population below 30 is about 5 per year (10 with the most conservative models). The acceptable threshold now is the the numbers of cancer occurring below 25: this is 8 cancer per year in Italy.

Conclusion

The Jury considered changing the screening protocols for girls vaccinated in their 12th year as appropriate. Tailored screening protocols based on vaccination status could be replaced by “one size fits all” protocols only when a herd immunity effect has been reached. Vaccinated women should start screening at age 30, instead of 25, with HPV test. Furthermore, there is a strong rationale for applying longer intervals for re-screening HPV negative women than the currently recommended 5 years, but research is needed to determine the optimal intervals. For non-vaccinated women and for women vaccinated in their 15th year or later, the current protocol should be kept.

References

Evaluating triage strategies: Risk stratification and thresholds, comparison of candidates

N. Wentzensen

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Background / Objectives

Triage of HPV-positive women is crucial to differentiate women at increased risk of precancer from women with transient HPV infections.

Methods

Many triage strategies are currently being evaluated, including cytology, HPV genotyping, p16/Ki-67 dual stain cytology, various methylation markers, and combinations of these markers.

Results

An ideal triage marker should identify the small group of women among the HPV-positives with high risk of precancer, while reassuring most women that risk of precancer is low. The evaluation of triage strategies needs to consider the primary screening approach and the setting (e.g. high-resource vs. low-resource; organized vs. opportunistic; physician collection vs. self-sampling). The absolute risk of precancer predicted by a triage test needs to be estimated in a population similar to which the assay will be used in. The risk thresholds, which determine what clinical action is taken given different assay results, may differ substantially between different settings. Consequently, it is important to compare different triage markers ideally in the same, but at least in very comparable populations with similar clinical management.

Conclusion

The presentation will introduce the design and rationale of the Improved Risk Informed HPV Screening (IRIS) Study that was designed to compare various triage strategies in a large HPV screening population.
MSS 03-02
IMMEDIATE TRIAGE AND RETESTING

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Background / Objectives

HPV positive women need triage. This usually includes an immediate triage test and repeat testing (commonly HPV) after some time in women negative to the first test. Objective was studying how the characteristics of the first test influence the second.

Methods

Data from the first survey of routine activity in Italy were used. In Italy HPV positive women have reflex cytology and are referred to colposcopy if it is ASC-US or more severe. If cytology is <ASC-US they repeat HPV after 1 year and are referred to colposcopy if still positive. Aggregated data (by local programme and 5-year age group) were collected on how many women were HPV positive, how many of them had abnormal cytology, were referred to colposcopy immediately or after 1 year and how many had a CIN2 or more severe histology detected during colposcopies performed because of abnormal cytology and because of persistent HPV positivity. The effect of the immediate referral rate to colposcopy (because of abnormal cytology) on the overall referral (either because of abnormal cytology or of persistent infection) was studied by regression. The same was done for the effect of local immediate referral rate and sensitivity of cytology (estimated as proportion of CIN2+ detected as a result of immediate colposcopy) on the overall local detection of CIN2+.

Results

Ten local programmes with complete data, having screened by HPV over 72,000 women were considered. The proportion of HPV+ women judged to have abnormal cytology varied strongly between local programmes, from 20% to 57%. Nevertheless such changes had very little effect on the overall referral to colposcopy and none on the overall detection of CIN2+. An increase of 10 absolute percent points (e.g. from 10% to 20%) in HPV positive women referred to immediate colposcopy resulted in only a 4.2% (95% CI 3.1 to 5.1) increase of the overall proportion of HPV positive women referred to colposcopy and in a 0.4% (-0.1 to 2.9) increase in the overall detection of CIN2+. An increase of 10 absolute percent points in the sensitivity of cytology resulted in a 1.1% increase (0.1-2.0) in the overall detection of CIN2+.

Conclusion

1-year HPV test repeat limits the effect of subjectivity in cytology interpretation. Most high-grade CIN missed by cytology are detected by HPV after one year. Sensitivity of the first triage test determines only the risk of invasive cancer before re-testing, given the re-testing interval. Increases in sensitivity of the first triage test can be exploited
by increasing the interval before repeat HPV. This would allow a greater proportion of HPV infection to clear, thus reducing the overall referral to colposcopy.
MSS 03-04
MOLECULAR TRIAGE AS PART OF CERVICAL SCREENING

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Background / Objectives

HPV based screening is slowly but steadily replacing cytology as the primary cervical screening test. It offers much higher sensitivity but lower specificity, largely due to transient infections with minimal progressive potential, so that some form of immediate triage on the same specimen is desirable to better identify those women who are most in need of direct referral to colposcopy. Where good cytology is available one option is reflex cytology. When this shows high grade changes, immediate referral for colposcopy is warranted, but lower grade cytological abnormalities still carry a high false positive rate even for HPV positive women.

Methods

A range of ‘molecular’ tests are now under evaluation to try to improve discrimination. Of these some form of HPV genotyping has been most fully investigated, but usually this has been limited to types 16 and 18. There is emerging evidence that fuller typing provides useful additional information and that types 31 and especially 33 carry a much higher risk that other types, and that types 39,56,59,66 and 68 carry lower risk and could usefully be considered ‘intermediate risk’ types. Types 18 and 45 do not have a high PPV for CIN2+, but are more related to invasive cancer and lesions in the endocervical canal, and deserve a different management with more emphasis on repeat testing to establish persistence. New evidence suggests that measures of viral load have type specific relevance and can add information about the likelihood of a high-grade precursor lesion. In addition measures of DNA methylation in both host and viral genes look promising, as does use of immunohistochemical and possibly RNA measures of p16, while tests of E6 and E7 protein levels still appear to lack sensitivity.

Results

Data on these markers will be reviewed from a range of different screening based studies.

Conclusion

Molecular testing has much to offer to improve efficiency of HPV related primary screening.
Evaluation of Use of Xpert HPV to triage women to ‘see and treat’ using Visual Inspection with Acetic acid (VIA) in rural Malawi.

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Background / Objectives

‘Screen and treat’ approaches to cervical screening offer the best hope for population-based screening in LMIC. Visual inspection with acetic acid (VIA) is recommended by WHO but is limited by lack of trained personnel and maintenance of quality skills. An objective test such as Hr-HPV detection as the first stage of screening would be beneficial.

Our objective was to assess the utility of Xpert® HPV as a primary screening tool in a rural setting where screening using VIA is the norm and an inexpensive intervention.

Methods

Following Malawian approvals and buy-in from regional and village chiefs, the Nkhoma cervical screening programme was established1. VIA providers attended Malawian Ministry of Health VIA course, supplemented by additional practical training in interpretation, treatment options and taking specimens for HPV testing. Laboratory staff performed Xpert® HPV according to manufacturer’s instructions using Preservcyt collection. Attempts were made to reduce the cost of HPV testing by reducing the volume of collection fluid, using alternative collection fluids and devices and also by introducing self-collection.

Results

Hr-HPV prevalence was established as 19.9% in 750 routinely screened women2. Hr-HPV ‘other’ was much more frequent than HPV 16 or 18/45, with HPV 31+ (P3) most commonly found. While lesions assessed as ‘suspicious’ cancers were nearly always Hr-HPV positive, there was considerable discrepancy between Hr-HPV positivity and VIA ‘positive’ results. These differences will be described in detail.

Conclusion

Xpert® HPV proved to be straightforward, had rapid turnaround and training was easily cascaded to more junior lab staff and to clinic staff. It is suitable as a point of
care test. Hr-HPV results were different from VIA results and considerable education is required to convince clinical providers that Hr-HPV testing is more specific. Steps could be taken to reduce costs of testing, particularly at the sample collection stage, but the cost per HPV test needs to be greatly reduced before it could be used as a primary screening tool in LMIC. At present, Nkoma CCSP could not afford to replace VIA same day 'screen and treat' with Hr-HPV testing.

References

REFERENCES


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MSS 04-01
Impact of change from cytology to molecular biology

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Background / Objectives

N/A

Methods

Although the value of molecular testing for nucleic acids of oncogenic HPV genotypes in primary screening for cervical cancer is now widely accepted, implementation of this screening technology remains slow. Only a few jurisdictions have taken the steps to adopt HPV testing as the anchor technology in cervical cancer screening. The most important challenges are fear of change, logistical complexities, and lack of proper health economic planning for programmatic changes in screening. Only cytology, with its 70-year history, is unequivocally linked to enduring reductions in cervical cancer incidence and mortality in resource-rich countries. However flawed as a technology, judged by the performance of today’s molecular-based counterparts, cytology screening is judged as an effective strategy that relies on a well-established workforce of trained cytotechnicians and cytopathologists.

Although the potential for improvement is substantial, there are many questions to be solved by implementation research, which will give policymakers confidence in making the changes from cytology to molecular testing and eventual incorporation of genotyping and biomarker-based staining of cytology smears. Is HPV plus Pap cytology cotesting, a policy widely adopted in the U.S., the only approach with sufficient safety for cervical cancer screening? Is HPV testing followed by cytologic triage a more cost-effective approach than cotesting? Is the inclusion of real-time partial genotyping during screening a cost-effective option, with or without cytology triage? Should HPV-based policies be reserved for women ages 25 (or 30) and older only? If so, what screening options should be recommended for younger women? Does the latter question matter, given that HPV vaccination is largely eliminating the high prevalence of HPV positivity among young women? Is self-sampling a solution to expand the coverage and bring equity to screening? US guidelines will gradually migrate to risk management based on a multiplicity of assay results providing more nuanced risk stratification. Would this serve as a universal solution across all high income countries? As HPV testing and ancillary molecular tools make inroads in cervical cancer screening, policymakers, physicians and healthcare payors cannot confidently decide among a bewildering array of strategies. Benchmarks of performance are urgently needed and realistic standards for regulatory approval must also be adopted to permit innovation and rapid deployment of validated molecular technologies.
Results
N/A

Conclusion
N/A

References
N/A
A European experience of implementation of HPV screening for CC

N. Van Der Veen, E. Brouwer, K. Goor, W. Rodenburg

none (Netherlands)

Background / Objectives

In 2011 the Health Council advised the Dutch Minister of Health, Welfare and Sport (VWS) that the current population screening for cervical cancer could possibly be improved. They advised to change the screening test from cytological screening to hrHPV screening. In reaction, VWS commissioned the RIVM Centre for Population Screening (RIVM-CvB) to perform a feasibility study. Based on the feasibility study, VWS decided to introduce hrHPV screening. The RIVM –CvB started implementing the new screening. This resulted in the new screening program that started in January 2017.

Methods

The feasibility study described the primary process, the organisation, the quality policy (including monitoring and evaluation) and the communication.(http://www.rivm.nl/Onderwerpen/B/Bevolkingsonderzoek_baarmoederkanker_voor_professionals). The study was performed involving all stakeholders. A three-year preparation phase was required that included drawing up the quality requirements, arranging the tendering procedures, adjusting guidelines, training and configuring the IT technology.

Results

The screening

Women receive an invitation with a folder to take a cervical smear. This is tested for hrHPV and cytology-triage. HrHPV+/ cyt + women are referred to the gynecologist, hrHPV+/cyt - for cytology after 6 month. HrHPV positive women receive a folder. This folder contains information about the hrHPV, if the virus can be prevented or treated, the relationship between the virus and sex, the consequences for pregnancy, and the results. Non-responders can apply for a self-sampling. Women aged 40, 50 and 60 who are hrHPV- are invited 10 years later. HrHPV+ or non-responders receive an invitation 5 years later.

Implementation of the screening tests

Five screening laboratories are involved in the new screening program based on the quality of the cytology, lower costs and sustainability (flexibility). Different acceptance tests were performed which showed that the HPV system performed comparable to the system at the time of clinical validation. Cytology training took place to prevent potentially unfavorable effects, such as a hrHPV-bias.
The tasks and responsibilities of the stakeholders and the requirements were specified and documented in the framework national requirements cervical cancer (http://www.rivm.nl/Onderwerpen/B/Bevolkingsonderzoek_baarmoederhalskanker_voor_professionals).

**Conclusion**

The new screening program is successfully implemented and the support of stakeholders is high. The organization is flexible to ensure fast introductions of improvements and innovations. The program is intensively monitored so that potentially unfavourable effects can be quickly adjusted.
What's happening in the second round?

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Background / Objectives

In Italy several pilot HPV screening programs started between 2011-2012 in early 2013 and provided guidelines for its application (stand-alone HPV testing by validated methods, cytological triage of HPV positives, beginning at age 30-35, 5-year intervals). The results of second round of HPV screening will be presented.

Methods

In 2012, 19 Italian organized cervical screening programmes from 10 regional programmes invited 311,856 women for HPV-based screening; 41.5% complied, with a decreasing North-South trend. All programs applied a cytology triage algorithm, re-inviting HPV+/cytology- women after one year to repeat HPV test. Here we present HPV positivity, cytology triage results referral rate and detection rate at baseline and in the second round of screening (after 3 years).

Results

The prevalence of HPV in the first round varies in different regions Italian while in the second round the percentage of HPV positivity is much more similar in various places; however, the positivity in the second round decreases by about 50% as well as the recall rate. CIN2+ detection rate in HPV+/cytology+ women ranged from 0.4/1000 to 0.8/1000 compared to a 6.5/1000-8.3/1000 range in first round.

Conclusion

Referral rate to colposcopy strongly reduced compared to first round (and lower than with cytology), therefore extra cost and anxiety for women limited to 1° round with HPV. Probability of carrying CIN2+ among HPV+ women strongly reduced after the first round of HPV screening. The DR total decreases for two reasons: there are fewer infections and why women with new infections still have a low number of lesions because the persistence of infection is for a few years, while at the first screening round are infections that are present from any years.

If there are fewer lesions in new infections in the second round you can change the triage strategies, much less aggressive or longer repeat interval. Effects expected (reduced) also with 5 year intervals HPV test positivity.
QUALITY ASSURANCE PROGRAMS FOR HPV-BASED CERVICAL CANCER SCREENING AND THE IMPLICATION FOR LABORATORY ORGANIZATION

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Background / Objectives

To appropriately evaluate and control the performance quality of HPV tests for major agreed-upon clinical indications, in addition to the appropriate guidelines we also need carefully designed and robust quality assurance programs. The number of HPV tests clinically validated for primary cervical cancer screening is increasing and appropriate quality assurance programs which can interrogate longitudinal robustness and quality are paramount.

Methods

Because the proper function of all testing steps (e.g., nucleic acid extraction, amplification and analysis of amplified products) determines the final clinical sensitivity and specificity of HPV testing, when designing quality assurance programs for evaluating and controlling the clinical performance of HPV tests one should produce panel members that mimic clinical samples as much as possible in terms of composition (human cells), transportation medium (with/without fixative), and total volume (greater than the minimum volume requested for pre-analytical processing). The main characteristics of a laboratory performing HPV-based cervical cancer screening and the personnel training needs to ensure an elevated quality of the entire process and the optimal use of the resources will be discussed. Additionally, the quality assurance, as both internal and external quality assessment systems, to be implemented and performed, as well as the description of the five major current HPV quality assurance programs (UK NEQAS, QCMD, DicoCare VEQ HPV-DNA HR, WHO HPV LabNet and Instand) and their advantages and limitations will be summarized and briefly commented.

Conclusion

Further international efforts are necessary that relevant standards, quality assessment programs, validation metrics and quality guidelines evolve to the level to suit the constantly changing nature of cervical cancer screening.
Background / Objectives

Primary HPV screening provides great reassurance for women who test negative that cervical cancer risk is very low. However, for women with positive HPV screening results, triage markers are needed to distinguish women at high risk of precancer who need referral to colposcopy from women with transient infections.

Methods

Currently, cytology is the primary triage test considered in HPV screening programs. Several new triage markers are currently being evaluated, including p16/Ki-67 dual stain cytology and host methylation markers.

Results

Development and evaluation of triage markers is one of the most important current tasks for improving cervical cancer screening programs.

Conclusion

In this presentation, the requirements for an ideal triage marker, and the required steps for evaluating such a marker, will be discussed and existing triage strategies will be evaluated in context with these criteria.
DEFINING THE POPULATION AT RISK: EPIDEMIOLOGICAL, GEOGRAPHICAL AND SOCIETAL MARKERS

S. Franceschi

International Agency for Research on Cancer (France)

Background / Objectives

Approximately 4.5% of all cancers worldwide (630,000 new cancer cases per year of which 570,000 cases in women and 60,000 cases in men) are attributable to HPV. Cancer risk and preventable fraction vary substantially by cancer site, sex, and age.

Methods

Comparison of the potential effectiveness of screening and vaccination approaches by cancer site and individual characteristics.

Conclusion

Of the six main cancer sites attributable to HPV, only for the cervix, and hence women, is screening recommended. Age is the only well-accepted criterion for screening eligibility. The typically recommended range is 25-64 years but it can be restricted, e.g., 30-49, to cope with resource limitations in low/middle income countries (LMICs), or expanded in some high-ICs with the aim of increasing women’s life expectancy. After the introduction of HPV testing, screening may be started later (30+ years) or stopped earlier thanks to anticipated diagnosis of precancerous lesions compared to cytology. Never-screened women will always be a priority. HIV infection and vaccination against HPV modulate screening intensity in opposite directions. Within screening, risk thresholds (benchmarks), based on HPV testing/genotyping, cytology, etc. are increasingly used in HICs to allow equal management of women for equal risk of CIN2+, irrespective of the screening tests. Only for anal cancer are lifestyle risk factors (men having sex with men and/or HIV+) very strong risk indicators. Even in high-risk men, however, anal cancer screening is however hampered by the low accuracy of HPV- and cytology-based risk stratification in anus. Worst of all, neither cytology, nor non-invasive HPV testing (in oral cells), allow screening for the relatively rare HPV-associated oropharyngeal cancer (80% in men). The detectability of oropharyngeal precancerous lesions is also uncertain. Finally, the efficacy of HPV vaccination to prevent vaccine-targeted HPV types and related lesions is best demonstrated in the female anogenital tract, but it is likely to apply to all HPV-related cancer sites in both sexes.

References


Cytohistologic Indicators of Cancer and Precancer Risk

M. Stoler

University of Virginia Health System (United States of America)

Background / Objectives

Before the epidemiology and molecular biology of cervical neoplasia were well established, cervical cancer screening was based on cytology alone. Cytology served as the basis for referral to colposcopy and colposcopic biopsy was the basis for therapeutic management. This morphology based system, repeated systematically effected a 75-90% reduction in cancer and precancer prevalence. Yet this approach has established limitations. The objective of this presentation is to review the contemporary risk estimates for CIN3+ of the major Bethesda categories and then to also provide similar estimates for the corresponding biopsy interpretations.

Methods

Morphologic images will be correlated with risk estimates based on the ALTS trial and contemporaneous epidemiologic literature. The error rates based on sampling issues and interpretive variability will also be explored based on literature data.

Results

The squamous cytologic categories of the TBS can be hierarchically ranked for CIN3+ risk in the order NILM>ASC-US>LSIL>ASC-H>HSIL. AGC associated risk is roughly the same as ASC-H and paradoxically mostly predicts for squamous lesions. HPV genotyping may modify risk substantially between categories. Interpretive variability is significant for both cytology (ASC-US) and Histology (CIN1 and CIN2). Sampling variability also confounds predictive value. These factors combine to prove that histology is not necessarily a superior predictor of biology compared to cytology.

Conclusion

Despite cytology and histology having established outstanding success in decreasing cervical cancer: A. Sampling issues and interpretive variability are highly confounding. B. Compensatory strategies that combine HPV molecular testing, biomarkers and better sampling methods are increasingly available. C. As prevalence decreases due to increased screening and vaccination these compensatory strategies will become increasingly necessary.
MSS 05-04
HPV GENETIC VARIATION

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Background / Objectives

Through an international collaborative effort, we have sequenced over 10,000 HR-HPV whole-genomes from well characterized epidemiological population cohorts. These NCI-HPV Genomics Project studies have uncovered several strong associations between HPV genetic variation and cervical carcinogenicity as well as new insights into HPV diversity in the population.

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 all HR-HPV types. We have HPV genome sequenced case and control cervical specimens from the NCI-KPNC PaP Cohort, U.S. SUCCEED study, and invasive cancers collected internationally by IARC for 5,570 HPV16’s, 1,729 HPV18’s, 1,002 HPV45’s, 2,000 HPV31’s, and 600 HPV35’s. For each HR-HPV type, we assessed variant sublineage and individual SNP associations with worst histologic outcome, and evaluated associations of the combined effects of rare nonsynonymous variants by viral gene region with risk of CIN3+.

Results

Specific HPV16 sublineages are strongly associated with histology-specific precancer/cancer risk, with an estimated relative risk of glandular lesions exceeding 100 for specific sublineages. The next most carcinogenic types also show variable risks of precancer/cancer for specific sublineages. Additionally, at a finer single nucleotide (SNP) level, we have identified many variable positions significantly
associated with precancer/cancer, and that controls have a significantly higher level of rare nonsynonymous variants in specific regions of the virus. For HPV16, an evaluation of rare SNPs determined that controls had a significant increase in rare variants consistent with APOBEC-induced nucleotide variations. E7 was strikingly more variable in the controls compared to the cases \((P=1.1\times10^{-7})\), and considerably more variable than E6. Controls also had increased rare variants in E1 \((P=0.001)\) and L1 \((P=7.9\times10^{-5})\).

**Conclusion**

These data indicate that HPV carcinogenicity is associated with genetic variation in specific viral regions.
MSS 05-06
HPV-Associated Cancers- Which Screening is the Best Approach?

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National Institutes of Health (United States of America)

Background / Objectives

HPV infections cause cancers at multiple sites, including the uterine cervix, the anal region, and the oropharynx among others.

Methods

Currently screening is only recommended for cervical cancers. This presentation will briefly review the evidence for and against screening of HPV-associated cancers at various sites and then focus on discussing different approaches to cervical cancer screening.

Results

There is not a single best strategy, instead, screening and management approaches need to be adapted to the specific setting and account for availability of technology, trained experts, and healthcare resources as well as for societal factors like risk tolerance and perception.

Conclusion

Examples for screening approaches from high- and low resource settings will illustrate these different strategies.
Viral methylation for predicting risk of ano-genital cancer

A. Lorincz

Queen Mary University of London (United kingdom)

Background / Objectives

We studied DNA methylation patterns of human papillomavirus (HPV) and tumor suppressor gene EPB41L3 in 148 anal and perianal biopsies to determine whether high levels of methylation would be associated with anal intraepithelial neoplasia (AIN).

Methods

DNA was extracted from tissue specimens and HPV genotype was determined by the PapType kit (Genera Biosystems Ltd). DNA was treated with bisulfite, amplified by PCR and levels of methylation were measured by pyrosequencing. Univariate and bivariate logistic models were fitted for statistically significant HPV and human genes with the outcome measures.

Results

The most prevalent HPV type was HPV16, detected in 54% of the 30 benign biopsies, 33% of the 43 low-grade AIN (lgAIN), 82% of the 59 high grade AIN (hgAIN) and 4 of the 5 anal cancers. A methylation score was developed (0.561*HPV16+0.439*EPB41L3) which had increasing values with severity of disease: the mean was 8.1% in benign, 13.2% in lgAIN, 22.3% in hgAIN and 49.3% in cancers (p<0.0001). The methylation score as a triage classifier at a cut-off of 8.8 gave a sensitivity of 90.6% (95% CI: 82.8, 96.9), specificity of 50.7% (95% CI: 39.7, 61.6) and area under the curve of 0.82 (95% CI: 0.75–0.89) for separating hgAIN and cancer from benign and lgAIN biopsies.

Conclusion

We conclude that methylation of HPV16 and EPB41L3 show highly significant association with increasing severity of AIN and cancer and may be useful as biomarkers in anal disease.
Simplifying histologic CIN grading based on the biomarker profile

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Background / Objectives

Accurate histological grading of cervical intraepithelial neoplasia (CIN) is essential for clinical management. However, CIN grading has a moderate inter- and intra-observer agreement. We investigated the reproducibility and the performance of a score system based solely on the cumulative score value of the biomarkers Ki-67 and p16ink4a (immuno-score).

Methods

The immuno-scores were compared to consensus pathologist CIN grading (3 pathologists) based on the combined interpretation of slides stained for H&E, Ki-67 and p16ink4a, and to individual CIN grading.

Results

The results show that the cumulative immuno-score (varying from 0-6) of a three tiered score for p16ink4a and Ki-67 staining has a higher reproducibility than the scores of the individual pathologists for CIN grading (H&E alone or combined with interpretation of immunohistochemistry). In addition, the accuracy of the immuno-score to predict underlying high-grade consensus pathologist CIN grade was at least as good as the individual pathologists CIN grading.

Conclusion

Thus, the selection of an area with abnormal cervical epithelium and subsequent separate assessment of the p16ink4a and Ki-67 immuno-score, results in a more reproducible performance of CIN grading compared to the classical way of grading. In the presentation the scientific background and the consequences of the use of this system for clinical practice will be highlighted.
MSS 07-01
Impact of the Scottish HPV vaccine programme on infection and cervical disease – a changing landscape

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Background / Objectives

In the UK, HPV vaccination of girls aged 12–13 as part of the school immunisation programme started in 2008 together with a three-year ‘catch-up’ programme for girls aged up to 18 years. Uptake rates for 3 doses in Scotland are high; almost 90% of girls eligible in the school programme and 65.5% in catch-up received all three doses. A national programme of longitudinal surveillance provides evidence on the impact and effectiveness of bivalent vaccine upon HR-HPV and CIN in routinely vaccinated girls and older catch-up women.

Methods

Until recently, Scotland began cervical screening at age 20 (though this was amended to the age of 25 in June 2016 to ensure consistency with other UK countries), and girls immunised as part of catch-up have been entering the screening programme since 2011. Thus, Scotland is almost uniquely placed to determine the impact of immunisation on cervical HPV infection, incidence of CIN, and genital warts in young women using national datasets, which can be linked effectively.

Results

Of the women born in 1989 of whom 0.3% were immunised, 29.2% (26.7-31.9) were HPV 16/18 positive aged 20 compared to 4.5% (3.5-5.7) of women born in 1995 (87% of whom were immunised). Only ~0.5% (0.26-1.1) of the 1995 cohort tested HPV 16/18 positive with the clinically validated assay, compared to 21.4% (18.9-24.0) of unvaccinated women born 1989-1992. Prevalence of HPV 31, 33 and 45 reduced from 14.2% (95% CI: 12-16.7%) in the 1988 cohort to 2.6% (95% CI: 1.9-3.6%) in the 1995 cohort. Vaccine effectiveness (VE) was higher in the younger cohorts with VE=54.8% (95% CI: 36.7-67.7%) in the 1990 cohort, increasing to VE=85.7% (95% CI: 78.7-90.4%) in the 1995 cohort, illustrating slightly lower but comparable 3-dose vaccine effectiveness for the cross-protective types to the vaccine types. Analysis of the catch-up cohorts suggest profound impact upon CIN 3, with effect most noticeable in more deprived women (rate of CIN 3 in 1988 cohort being 11.9 per 1000 py (10.4-13.4) vs 2.9 per 1000 py (2-3.9) for the 1994 cohort.

Conclusion

HPV 16,18,31,33 and 45 are implicated in 84% of invasive cervical cancers and in Scotland these 5 HPV types account for 90% of cancers. For all 5 types, vaccine effectiveness in the 1995 cohort exceeded 80%, differing from recent meta-analysis
which found evidence for HPV 31 cross-protection but little evidence for reductions of HPV 33 or 45. Encouragingly, there was no significant increase in non-16/18/31/33/45 HR-HPV types even though 16/18 prevalence has reduced by 6-fold (28.9% to 4.8%). Bivalent HPV immunisation has the potential to prevent up to 90% of infections that cause cervical cancer, with effects most notable in deprived women.
MSS 07-05
Understanding changes in non vaccines types

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Background / Objectives

Monitoring changes in non vaccine HPV types following the introduction of HPV vaccination is important to provide information on cross-protection and potential type-replacement.

Methods

We conducted a systematic review and meta-analysis to consider changes in the prevalence of non-vaccine HPV types in other studies comparing type-specific HPV prevalence between pre-vaccination and post-vaccination periods. Results were stratified by age-group (≤19 years old and 20-24 years old).

Results

The meta-analysis included data from 9 studies. There was evidence of a decrease in HPV31 in the younger age-group (≤19 years old) but little evidence of a reduction in HPV33 or HPV45. Results were heterogeneous for HPV31, HPV33 and HPV45 in 20-24 year old women. There was some evidence of increases in some high-risk HPV types although these results were inconsistent for the two age-groups and the vaccine used.

Conclusion

We found evidence of cross-protection against some closely-related types. However, there was no clear evidence of type replacement; increases in non vaccine types could be due to other factors such as changes in study populations or an unmasking effect of broad spectrum assays. Continued monitoring of these HPV genotypes remains important. This presentation will also summarise more recent data on changes in non-vaccine HPV types following the introduction of HPV vaccination.
THE BURDEN OF CANCER CAUSED BY HPV INFECTION: WOMEN AND MEN

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

International Agency for Research on Cancer (France)

Background / Objectives

HPV is the cause of almost all cervical cancer and is responsible for a substantial fraction of other anogenital cancer and oropharyngeal cancer. Understanding the HPV-attributable cancer burden can boost programs of HPV vaccination and HPV-based cervical screening.

Methods

Attributable fractions (AF) and the relative contributions of different HPV types were derived from published studies reporting on the prevalence of transforming HPV infection in cancer tissue. Maps of age-standardized incidence rates of HPV-attributable cancers by country from GLOBOCAN 2012 data are shown separately for the cervix, other anogenital tract, and head and neck cancers.

Results

4.5% of all cancers worldwide (630,000 new cancer cases per year of which 570,000 cases in women and 60,000 cases in men) are attributable to HPV. The HPV AF is 8.6% in women, with vast variations by region, and 0.8% in men. AF in women ranges from <3% in Australia/New Zealand and the USA to >20% in India and sub-Saharan Africa. Cervix accounts for 83% of HPV-attributable cancer, two-thirds of which occur in less developed countries. Other HPV-attributable anogenital cancer includes 8,500 vulva; 12,000 vagina; 35,000 anus (half occurring in men); and 13,000 penis. In the head and neck, HPV-attributable cancers represent 38,000 cases of which 21,000 are oropharyngeal cancers occurring in more developed countries. Contrary to the other cancer sites, the majority of HPV-attributable cancer of the head and neck occur in men (80%) and incidence rates are larger in high-income than low/middle-income countries.

Conclusion

Economic development is not sufficient to reduce the burden of sexual transmission of HPV and the related cancer burden unless population-based interventions are prioritized and made cost-effective in low/middle income countries. Universal access to vaccination is logistically less demanding than cervical screening and is therefore the key to avoiding most cases of HPV-attributable cancer in both sexes. The preponderant burden of HPV16/18 and the possibility of cross-protection emphasize the importance of the introduction of cheaper vaccines in less developed countries, even if only HPV16/18 vaccines are affordable.
References

MTC 01-02
UNDERSTANDING THE EPIDEMIOLOGY OF HPV INFECTION: THE GLOBAL VIEW

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Background / Objectives

In the past decade research has clearly demonstrated that HPV causes cancer in men and women at multiple anatomic sites. While the natural history of cervical HPV infections is well understood following decades of research globally on this one anatomic site, there have been far fewer studies examining the natural history of HPV at the anal canal and oral epithelium in men and women, penile epithelium in men, and vulvar and vaginal epithelia in women.

Methods

Often mirroring cancer incidence rates, HPV prevalence and infection natural history varies by gender and anatomic site of infection. Among men, penile HPV prevalence is high at ~50% with remarkable consistency across the countries reporting prevalence at this anatomic site. Penile or external genital HPV infections in men are consistently higher than observed among women of similar countries. Overall, anal HPV prevalence is relatively low at ~10% in the general male population but fold higher when examined among men who have sex with men (MSM). Oral HPV infection is rare (~5%) and significantly differs by gender whereby males have significantly higher prevalence compared to females.

Conclusion

More research is needed to characterize HPV natural history at each anatomic site where HPV causes cancer in men and women, information that is critical to inform the basic science of HPV natural history and to the development of future infection and cancer prevention efforts.
Emerging issues on HPV transmission, focus on differences by sex

G. Dsouza

Johns Hopkins Bloomberg School of Public Health (United States of America)

Background / Objectives

Invited Talk.

This talk will review differences in HPV infection risk between men and women. Risk of genital HPV, anal HPV, and oral HPV will be discussed and compared between men and women.

Methods

N/A

Conclusion

It has been noted 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. Recent data will be reviewed which help to explain these differences in oral HPV infection between men and women.
The growing national HPV-based screening program strategies

C. Meijer

department of Pathology, VUMC, Amsterdam

Background / Objectives

For session

MTC 02

Cervical cancer control in high income countries - current standards and challenges

Cytology has been for a long time the primary screening tool in cervical cancer screening programmes. The recognition that the sensitivity for cervical (pre)cancer of an HPV test is much higher than the sensitivity of cytomorphological investigation of a cervical smear and that an objective HPV test is more easy to implement than a subjective cytology test has led to the introduction of HPV testing as primary screening tool in cervical screening programmes. Introduction of an HPV test in population based screening raises questions about “which HPV test and which triage test should be used?, which screening intervals should be advised?, should we use self-collected cervico/vaginal material? and how should we follow up women who have been vaccinated. Although recently guidelines for HPV based screening have been developed questions about a number of the above mentioned issues still remain. Of course the burden of the disease, local setting and the resources available in different countries determine the screening strategy that will be implemented. One way to limit the growing national HPBV based screening strategies is to define minimum requirements in terms of efficacy and safety of the screening programme in relation to the burden of investigations for women participating in the programme but do not have cervical (pre)cancer. In the presentation these issues are further highlighted.

Methods

For session

MTC 02

Cervical cancer control in high income countries - current standards and challenges

Conclusion

For session
MTC 02

Cervical cancer control in high income countries- current standards and challenges
BARRIERS AND OBSTACLES OF HPV SCREENING: ADDRESSING THE SOLUTIONS

J. Smith

University of North Carolina, Chapel Hill (United States of America)

Background / Objectives

Invasive cervical cancer (ICC) is a cancer of disparities, with higher incidence and mortality rates among black women as compared to white women and Hispanic women as compared to Non-Hispanic women in the U.S. Early detection and treatment of cervical precancerous lesions can dramatically reduce the incidence of ICC, but cervical cancer screening coverage is inadequate. Insufficient screening is the largest factor in reducing ICC: an estimated 56% of incident ICC in the U.S. is due to insufficient screening, 32% to detection failure, and 13% to follow-up failure (1). Low socioeconomic status is associated with lower screening rates and may limit a woman’s ability to access preventive care due to resource-related barriers. The Affordable Care Act (2010), has the potential to increase cervical cancer screening rates by expanding insurance coverage and requiring provision of free screening services. However, Medicaid expansion has not been implemented in all states, and only an estimated 63% of Medicaid-eligible adults participate in Medicaid.

At-home HPV self-collection has the potential to increase cervical cancer screening completion among under- and never-screened women. HPV self-collection is a technique by which a woman uses a simple collection brush to obtain cervico-vaginal cell samples to test for infection with high-risk HPV infection, an objective indicator of elevated risk for cervical cancer. Self-collection for HPV testing compares favorably in sensitivity and specificity to that of physician-collection for the detection of HPV infection and high-grade cervical lesions. Only one U.S. study has evaluated the effect of at-home HPV self-collection on screening uptake, distributing kits door-to-door through community-based recruitment (2). Several European studies have found that offering at-home HPV self-collection to under- and never-screened women via direct mailing leads to higher screening completion compared to mailed invitations for in-clinic screening (e.g. 30.8% via self-collection vs. 6.5% via reminders in the Netherlands, p<0.001) (3). These studies found relatively high rates of follow-up for in-clinic Pap testing among women receiving HPV positive self-collection results (e.g. 86% in a Dutch study) (4), and that use of at-home HPV self-collection kits led to higher detection of CIN2+ than mailed written reminders alone.

Our My Body My Test studies in North Carolina show that at-home HPV self-collection kits delivered via mail are highly acceptable to under-screened, low-income women (69% to 85% return rates), consistent with high HPV self-collection acceptability demonstrated in other states and countries.

Methods

N/A
Results
N/A

Conclusion
N/A

References
[1. Leyden WA et al. JNCI. 2005;97(9):675-83.]
The transition era of HPV vaccination, form the previous to the new generation of HPV vaccines

E. Joura

Medical University Vienna (Austria)

Background / Objectives

The first generation of HPV vaccines, the quadrivalent HPV 6/11/16/18 and the bivalent HPV 16/18 vaccines were licensed in Europe in 2006 and 2007 respectively. In 2015 a ninevalent HPV 6/11/16/18/31/33/45/52/58 vaccine was licensed.

Methods

A review of the current status of data and licensure and the transition process in some European countries will be evaluated. Considerations for those already fully or partially vaccinated will be described.

Conclusion

In the US, where the ninevalent HPV vaccine was available already at the beginning of 2015 the transition was almost accomplished in one year. For Europe it was mandatory to have data and results on the 2-dose schedule, therefore the vaccine became available in May 2016. At this time the vaccine was licensed by EMA for females and males from 9 without any upper age limit, and for the 2-dose schedule. Every cycle started with the quadrivalent or bivalent vaccine could be accomplished with the ninevalent vaccine, however to get the full protection against the 5 new HPV genotypes a full course of 2 or three doses (depending on age) has to be given.

References


2. Van Damme P, et al. Use of the nonavalent HPV vaccine in individuals previously fully or partially vaccinated with bivalent or quadrivalent HPV vaccines. Vaccine 2016;34:757-61.

ACCUMULATING EXPERIENCES ON THE REAL LIFE, USE OF CARE HPV

G. Clifford

International Agency for Research on Cancer, Lyon (France)

Background / Objectives

The Bhutanese Screening Programme recommends a Pap smear every three years for women aged 25–65 years, and coverage ranges from 20% to 60%, being especially challenging in rural settings. The “REACH-Bhutan” study was conducted to assess the feasibility and outcomes of a novel approach to cervical cancer screening in rural Bhutan based on the careHPV test on self-collected samples.

Methods

Cross-sectional, population-based study. Women were recruited in rural primary health care centres, i.e. Basic Health Units (BHU), across Bhutan. Overall, 3,648 women aged 30–60 were invited from 15 BHUs differing in accessibility, size, and ethnic composition of the population. Participants provided a self-collected cervico-vaginal sample and were interviewed. Samples were tested using careHPV in Thimphu (the Bhutanese capital) referral laboratory. Main outcome measures were screening participation by geographic area, centre, age, and travelling time, as well as previous screening history and careHPV-positivity by selected characteristics of the participants.

Results

In April/May 2016, 2,590 women (median age: 41) were enrolled. Study participation was 71% and significantly heterogeneous by BHU (range: 31%–96%). Participation decreased with increase in age (81% in 30–39 year-old women; 59% in ≥50 years), and travelling time (90% in women living <30 minutes from the BHU versus 62% among those >6 hours away). 50% participants reported any previous screening. 265 women (10%; 95%CI 9%–11%) were careHPV-positive, with a significant variation by BHU (range: 5%–19%) and number of sexual partners (prevalence ratio for ≥3 vs. 0–1=1.55; 95% CI: 1.05–2.27). On the technical side, although the central laboratory in Thimphu was able to deliver careHPV results to each BHU in a median of 11 days, the careHPV platform turned out to be less reliable than hoped and, even after completion of the initial training period, there still continued to be substantial wastage due to invalid careHPV runs. In addition, the original plan of rapidly recalling women and offering them cryotherapy in each BHU was hindered by difficulties in transport and/or malfunction of cryotherapy equipment.

Conclusion

Community-based cervical cancer screening, by testing self-collected samples for careHPV, can achieve high coverage in rural Bhutan. Nevertheless, implementation
was characterised by logistic and technological challenges both with careHPV and treatment technology.
MTC 03-05
SELF-SAMPLING EXPERIENCE FROM SCOTLAND TO MALAWI AND BACK.

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Global Health Academy, University of Edinburgh, Edinburgh, UK. (United kingdom)

Background / Objectives

Malawi has the highest incidence of cervical cancer in the world. The practical and sustainable ‘screen and treat’ programme has been introduced in the Nkhoma region based on VIA and thermo-coagulation for treatment of early lesions(1). Here we present our experience with HPV testing of women presenting for VIA using self-collected vaginal samples and Xpert HPV (Cepheid) test. We have previously (2015) clinically validated the self-sampling in Scottish cervical screening hence had confidence in introducing it to Malawi. We aimed to establish feasibility of self-vaginal sampling and compare HPV prevalence of different collection protocols / devices / media with a view to validation of a cost-effective solution for low-income countries.

Methods

Provider-taken cervical and self-collected vaginal specimens were obtained from women attending routine VIA clinics in Nkhoma Hospital and associated Health Centres. The cervical HR-HPV prevalence was established(2). Women provided self-collected specimens in the clinic following verbal and diagrammatic instructions. Collections using swabs suspended with 5ml of Preservcyt (PC) or saline were carried out in June 2015. During 2016, Quintips® and Vibabrush devices were trialled for self-collection with 4-5ml of PC being added.

Results

Women were open to HPV testing provided sufficient information had been given. Cervical HR-HPV prevalence was established as 19.9% (2). HR-HPV “other” was much more frequent than HPV 16 or 18/45, with HPV 31+ (P3) most commonly found. Self-collected specimens using swabs in PC showed the highest HR-HPV positivity. However, when saline was used as the medium, the prevalence was lower (21.4%). The comparison of HR-HPV detection obtained with self-collected vaginal samples using swabs, Quintips and Vibabrushes resulted in 29.6%, 26.9% and 18.1% detection respectively. No invalid specimens were obtained with either swabs or Quintips when suspended in PC.

Conclusion

Three self-sampling methods offered were universally acceptable. All but Vibabrush self-sampling resulted in higher HR-HPV detection in comparison to cervical sampling. Dry vaginal swab suspended in 5 ml of saline within less than 2 hours from collection had the closes to cervical sampling detection rate. The HR-HPV detection
with Xpert® HPV is straightforward, has rapid turnaround and should now be validated with larger numbers using self-taken vaginal samples and low cost collection systems in LMIC.

References

2. Cubie HA, Morton D, Kawonga E et al. JCV 2017; 87:1
Existing data on single-dose regimens of the prophylactic HPV vaccines

A. Kreimer

US National Cancer Institute (United States of America)

Background / Objectives

HPV vaccines were licensed and recommended a decade ago to reduce individual- and population-prevalence of HPV, a necessary cause of cervical carcinogenesis. These vaccines were initially tested and approved in three-dose regimens. Vaccine uptake has been poor in many world regions, likely the consequence of high costs and the intensive infrastructure required for administering three doses over a six-month period. In time, serological data provided consistent evidence that two doses administered among adolescents at least six-months apart evoked immunological responses that were non-inferior compared to three doses among the 16-to 26-year-old women who experienced protection in clinical trials. Consequently, European and US authorities reduced the dosing recommendation for adolescents to two doses. Yet, the Costa Rica Vaccine Trial (CVT) and the PATRICIA trial, both of which tested the bivalent HPV vaccine, showed in post-hoc analyses similar vaccine efficacy over four years among women who received one, two and three doses of the HPV16/18 vaccine, and stable antibody responses; the CVT has recently extended these data to seven years. Additionally, the 36-month preliminary analysis of a post-licensure trial of the quadrivalent vaccine showed similar protection against cervical infection with HPV16/18 regardless of number of vaccine doses. In this talk, post-hoc data from phase 3 clinical trials on efficacy and immunogenicity of a single dose of the HPV vaccines will be discussed.

Methods

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Results

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Conclusion

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References

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Background / Objectives

While prevention of most female specific cancers (ovarian, breast, endometrial) has not progressed substantially in recent years, significant progress has been made with cervical cancer due to the accessibility of the cell of origin (cervical smear) and availability of a test for a causal agent (human papilloma virus); together these enable identification of high risk individuals and interventions in order to prevent infection or halt the progression to invasive cancer.

Our FORECEE (Female cancer prediction using cervical omics to individualise screening and prevention) consortium has developed an exciting opportunity to utilise clinically abundant cervical cells in tandem with a multi-omics enabled (genome, epigenome, metagenome) analysis pipeline to understand an individual’s risk of developing all female specific cancers and to direct a personalised screening and prevention strategy. Cervical cells — currently collected within cervical cancer screening — provide an ideal window into other female specific cancers because
they are (i) an excellent non-invasive source of high quality DNA, (ii) provide a readout for environmental exposure, (iii) are part of the Muellerian tract and (iv) are hormone sensitive, recording (via the epigenome) various hormonal conditions over a lifetime that trigger cancer development.

Methods

Our consortium comprises a multi-disciplinary team of experts in clinical oncology, risk-benefit communication, omics technologies, decision analysis, health economics and public health. We will examine the effectiveness of the proposed cervical cell omics analysis method and investigate the legal, social, ethical and behavioural issues related to the implementation of the risk prediction tool, through direct interaction with stakeholder groups with a view to ensuring its rapid translation into clinical practice across Europe.

Conclusion

The FORECEE project is aligned with the novel concept of "P4 Medicine" (predictive, preventive, personalised, and participatory): it aims to develop a risk prediction tool and translate its output into personalised recommendations for screening and prevention of female cancers.
The Clinical Value of Extended HPV Typing

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Background / Objectives

HPV based screening relies on assays that typically detect a pool of 13-14 HPV genotypes associated with cervical cancer. However, the oncogenic potential varies substantially between these types, with HPV16 and HPV18 accounting for over 70% of all cervical cancers, while several other types rarely cause cancer.

Methods

Several currently available HPV assays separately detect HPV16/18, and sometimes HPV45, to allow for better risk stratification of a positive HPV result compared to a pooled assay. In some settings, women with HPV16/18 positivity are immediately referred to colposcopy, while women positive for other carcinogenic types are triaged with cytology.

Results

Recent data from large population-based studies suggest that extended HPV genotyping beyond HPV16/18 may be useful in some situations. The remaining HPV types can be separated into two major risk groups, one group that includes HPV31, 33, 45 and several others with intermediate risk and one group, including HPV39, 51, 56 and several others with very low risk.

Conclusion

This separation can allow for more refined risk stratification, particularly in women with minor abnormalities, and could reduce colposcopy referral of women at very low risk.
Molecular markers for risk-stratification of HPV-positive women

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Background / Objectives

Cervical cancer is associated with a persistent infection with high-risk HPV and develops through precancerous lesions (high-grade cervical intraepithelial neoplasia; CIN2/3). In CIN2/3 the normal viral life cycle is aborted and the viral oncogenes E6 and E7 are overexpressed in proliferating cells. This results in the induction of (epi)genetic changes that drive progression to cancer. With the introduction of primary hrHPV testing in cervical screening, these host cell changes provide promising markers for the management of hrHPV-positive women. These so-called triage markers ideally identify hrHPV-positive women with clinically relevant high-grade CIN lesions in need of treatment (“advanced CIN2/3”).

We set out to discover and validate DNA methylation markers that can be used for triage of hrHPV-positive women.

Methods

Targeted and genome wide methylation discovery screens were performed on hrHPV-transformed cell lines and cervical tissue specimens. Candidate genes targeted by DNA methylation were validated by methylation-specific PCR (MSP) on cervical exfoliated cells.

Results

We identified a series of candidate methylation target genes, of which the methylation levels showed a significant increase with severity of cervical disease (p<0.005). Analysis of HPV-positive cervical scrapes and self-collected cervico-vaginal specimens showed that these methylation markers enable the detection of all advanced CIN2/3 lesions and cervical cancers in both HPV-positive scrapes and self-collected specimens.

Conclusion

DNA methylation analysis provides an attractive triage tool for hrHPV-positive women, which is particularly useful for self-collected specimens. Triage by DNA methylation analysis specifically detects cervical lesions in need of treatment and can prevent overtreatment of non-progressive lesions.
GLOBAL OVERVIEW OF COMMERCIALY AVAILABLE HPV TESTS

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Background / Objectives

Testing for high-risk HPVs is an invaluable part of clinical guidelines for cervical carcinoma screening, management and treatment.

Methods

As of May 2017, at least 210 distinct commercial tests for detection of alpha HPVs and at least 150 variants of the original tests are available at the global market. Unfortunately, only a subset of commercial HPV tests has documented clinical performance for agreed indications for HPV testing in current clinical practice. For more than half of HPV tests on the global market, no single publication in peer-reviewed literature can be identified. In contrast to commercial kits for “classical” molecular microbiology targets, the great majority of HPV tests currently on the market does not contain sample extraction part and number of them don’t even mention recommended nucleic acid extraction methodology in their manufacturer’s instructions. Only a minority of HPV tests on the market have internal controls.

Conclusion

Manufacturers of HPV tests are urged to put more effort into evaluating their current and future products. Manufacturers should seek advice from established HPV researchers in the very early phase of development on: (i) how to properly design a novel HPV test; (ii) to define intended use of future test (clinical, epidemiological, for research only) and (iii) how to evaluate test performance in such a way that the HPV community will accept evaluation/validation results. Since extraction of nucleic acids is an invaluable part of the whole HPV testing procedure we urge manufacturers of HPV tests to put substantially more effort into this initial and crucial step of molecular testing. Manufacturers should validate sample extraction procedure for each of the recommended sample collection devices and clinical sample types and list of validated sample collection devices and specimen types should be provide in the manufacturer’s instructions. Manufacturers’ independent evaluations and publication of results in peer-reviewed journals are also crucial. We predict that the number of commercial HPV tests will continue to increase in the near future, due to promising marketing opportunities. Namely, in contrast to “classical” molecular diagnostic microbiology testing areas which are considered very mature with expected annual growth rates of 2-5%, annual growth rates of HPV tests selling are expected to remain as high as 20% at least through 2020.
Quality control requirements in primary HPV screening

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Background / Objectives

A change from cytology to HPV testing in primary cervical cancer screening implicitly changes the requirements for quality control and quality assurance compared to the current cytology based screening.

For molecular HPV screening, quality control is not only required for the actual screening test, but will also be required for bio-banking of cervical screening samples with respect to audit and quality assurance of the overall screening program performance. Quality control requirements will -to a large degree- be defined by choice of sampling medium and by choice of HPV assay. But how to design quality control measures on molecular HPV cervical screening activities?

The presentation will focus on 1) the quality of liquid based cytology medias, ThinPrep and SurePath, for use with molecular HPV assays, 2) tools to quality control HPV based screening in individual laboratories and in series of laboratories, 3) describing the impact of assay specific cross reactivity to non-targeted HPV genotypes, and the challenges with respect to false-positive screening results when using HPV assays with bulk detection of many genotypes. Finally, the presentation will take a look at the biobanking requirements for longitudinal quality control and assurance for audit purposes. The latter is expected to be a large and different work task compared to storing cytology slides for quality assurance.

Methods

N/A

Results

N/A

Conclusion

N/A

References

Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study.


Quality assurance of human papillomavirus (HPV) testing in the implementation of HPV primary screening in Norway: an inter-laboratory reproducibility study.

Engesæter B et al. BMC Infect Dis. 2016
SS 01-04
HPV TESTING REQUIREMENTS FOR ORGANIZED CERVICAL CANCER SCREENING PROGRAMMES

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Background / Objectives

On Sept. 15th 2016, a directive for the upcoming organized Cervical Cancer Screening Program in Germany was issued. Women from the age of 35 years will be offered co-testing by an HPV test in combination with cytology every three years. Younger women still have annual PAP smears. New requirements for an HPV DNA test in an organized population based screening setting should be as follows – the test shall be able to detect only the 13 HPV types classified by the IARC/WHO as carcinogenic to humans. The clinical sensitivity and specificity of an HPV test for the detection of CIN2+ must not be lower than 95% and 98% of already established and in RCTs used HPV test systems, such as the HC2 (QIAGEN) test, respectively. Furthermore the test-positivity rate in women with NILM should not be higher than that of the HC2 test. Intra- and inter-laboratory reproducibility on different instruments and with different staff should be at least 90%. And because all screening programmes that include HPV testing comprise screening intervals of at least 2-3 years, the longitudinal cumulative incidence rate of CIN2+ and the NPV should not be significantly different from the HC2 test used in European RCTs. In addition, the desired test should have demonstrated convincing performances in pilot trials, should be accepted by the lab and the referring ObGyn and should be cost-effective.

Methods

In the past years, we have performed three HPV DNA and RNA test comparisons (Abbott: realtime high risk HPV test, Hologic: CERVISTA, APTIMA) in cross-sectional studies (N= approx. 10,000 each) based on routine screening populations and currently perform one new RNA test comparison (APTIMA; N=9451) in a long-term prospective (5 years) routine screening cohort.

Results

Although all tests revealed a high degree of automatisation, inter- and intra-laboratory reproducibility and non-inferiority in the clinical performance to the gold standard, other features need to be considered as well. Tests like CERVISTA revealed a twice as high positivity rate in women with normal cytology than the HC2 test leading to an increased referral rate in our comparative cross-sectional study. The Abbott HPV test missed two cases of HPV31-positive CIN3. In contrast, the APTIMA RNA-based test consistently shows a comparable clinical sensitivity to HC2 in combination with a higher clinical specificity. This reduces the number of follow up procedures by 23% in a screening program while at the same time keeping a high sensitivity for the detection of CIN2+. 
Conclusion

Prior to their introduction into nationwide screening programmes, HPV tests need to be evaluated in pilot studies under real screening conditions.
SS 01-05
NEXT GENERATION OF HPV TESTING: FROM GENOTYPING TO MOLECULAR MARKERS

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Background / Objectives

High-risk HPV infections are known to cause cervical and anal precursor lesions varying from productive infections that are likely to regress, to advanced transforming infections that might lead to cancer. In order to differentiate between these two types of lesions, different molecular markers that focus on human gene expression patterns or viral gene expression patterns are used. This study aims to provide insights in the value of markers that detect: (1) changes in human gene expression, hypermethylation of tumour suppressor genes in cervical smears, and in (2) viral gene expression patterns, immunohistochemical (IHC) staining with HPV E4 and p16INK4a in cervical and anal biopsies.

Methods

Women who underwent LEEP treatment for the suspicion of a high-grade CIN lesion (CIN2+) and had a cervical smear suitable for methylation testing were selected from a follow-up study conducted in Delft, The Netherlands and Barcelona Spain (N=199). Cervical smears were tested for hypermethylation of the tumour suppressor genes CADM1, MAL and miR124-2. Methylation results were compared to biopsy diagnoses. Cervical biopsies were stained with primary antibodies HPV panE4 and p16INK4a and immunostainings were scored to identify productive lesions (HPV E4+) and transforming lesions (HPV E4-, p16≥2/3 of the epithelium). In addition, the same IHC staining’s were performed on a selection of 67 anal biopsies collected in New York, USA. Results were compared with previously generated research.

Conclusion

In both cervical and anal lesions, the combination of HPV E4/p16 immunostaining assists in distinguishing between different stages of lesion development and disease severity. E4 expression is associated with low-grade disease and the use in combination with p16 might allow us to divide the cervical and anal intraepithelial neoplasia grade 2 (-IN2) group into E4 expressing lesions which are more low-grade-like and E4 negative lesions which are more high-grade-like. Hyper methylation detected in a cervical smear correlates to the worst lesion found on biopsy, with >70% of CIN3+ lesions positive for hyper methylation of at least one of the tested tumour suppressor genes. The relation of immunohistochemical markers tested on biopsies and methylation markers tested on smears to identify advanced transforming high-grade lesions is important for the identification of patients suitable for treatment.
HPV testing in urine and the possible applications

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Background / Objectives

From a practical point of view urine samples have, compared to clinician-collected cervical samples, a number of benefits. They are non-invasive, suited for self-sampling at home, and serial sampling is possible. It is important to understand the rational of detecting potential genital infections/markers in urine. Indeed, like all the epithelial tissues, the superficial cell layers of the genital track are subject to continual exfoliation. This means that debris from exfoliated cells from matrix, cervix and vagina mix with the vaginal fluid. This cervico-vaginal-fluid further accumulating between the small labia and around the urethra opening contaminates the first voided, i.e. initial stream of urine. This hypothesis has been confirmed via different routes. Also the use of a DNA preservative has been shown to have major impact on the analytical sensitivity. We will discuss the use of different assays on urine samples as well as settings in which urine has already been used successfully.

Methods

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Results

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Conclusion

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UNDERSTANDING AND MITIGATING THE PSYCHOLOGICAL IMPACT OF HPV DNA TESTING ON WOMEN

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Background / Objectives

As more and more countries move to using primary HPV DNA testing in cervical screening, there is an increasing need to understand the psychological impact this change may have on women taking part in screening. A number of studies have found that testing positive for a sexually transmitted virus in the cancer screening context can be confusing and distressing, raising issues of stigma and shame which are less common responses to abnormal cytology results. This paper will describe the protocol of a study in England aimed at assessing psychological responses to different screening results in the context of primary HPV testing.

Methods

The Psychological Impact of Primary Screening (PIPS) study is a cross-sectional observational study being carried out in the context of pilot HPV primary screening in England. The study uses validated measures of anxiety, distress and quality of life to compare women receiving 6 different screening results: cytology negative (control group); HPV negative; HPV positive with normal cytology; HPV positive with abnormal cytology; persistently HPV positive with normal cytology; and HPV negative following a previous HPV positive result. Questionnaires are being used to assess a range of psychological constructs 2 weeks, 6 months and 12 months after women have received their screening results. Between-group differences and changes over time will be explored.

Conclusion

The PIPS study and other similar research will shed light on the psychological challenges faced by women having primary HPV testing and will point to ways in which communication strategies can be better designed to minimize adverse psychological responses. Measuring quality of life in such studies allows any adverse impact of HPV positive results to be included in health economic modelling of new screening algorithms. This paper is part of the session on “Identifying and Overcoming Communication Challenges”.

COMMUNICATION ABOUT HPV VACCINATION BY HEALTH CARE PROVIDERS: A SUMMARY OF RESEARCH AND GOOD CLINICAL PRACTICE

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Background / Objectives

Some countries have achieved high rates of HPV vaccination by administering the vaccine in school settings. This school-located approach minimizes, but does not entirely eliminate, the need for direct conversations about vaccination with a health care provider (HCP). Other countries, including the U.S., largely rely on office-based HPV vaccination, in which HCP communication with parents and adolescents are central to vaccine acceptance. However, research suggests that a primary reason for non-vaccination is the lack of a strong recommendation by HCPs. This presentation will be part of the session “Identifying and Overcoming Communication Challenges.”

Methods

A review of research literature on HCP communication about HPV vaccination, as well as reviews of clinical training programs to improve such communication, will form the basis of this presentation.

Results

Research shows that many HCPs are ineffective at communicating about HPV vaccine. They infrequently make a strong, presumptive recommendation, often recommend delay of vaccination, and at times engage in long, overly detailed, unproductive monologues. Intervention research points to advantages of presumptive recommendation approaches. Training programs also emphasize strong, presumptive recommendations, but also focus on ways of engaging HPV-vaccine-hesitant parents and adolescents.

Conclusion

Research and good clinical practice support HCP communication about HPV vaccination that begins with a strong, presumptive, same-day recommendation. Most parents and adolescents will respond well to this kind of communication approach and will accept vaccination. For parents and adolescents who push back against this approach, it is important to implement strategies that engage with, and respond to, the hesitancy. These strategies may include use of motivational interviewing techniques and provision of accurate information about HPV vaccine safety and effectiveness.
APPROACHES FOR RESPONDING TO AND MINIMIZING NEGATIVE PUBLIC HEALTH POLICY CHANGES RELATED TO HPV VACCINATION

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Background / Objectives

Communities around the world have experienced HPV vaccination initiatives differently. While some have accepted vaccination programs and their scale-up, others have faced issues along the way. How these issues are addressed at community levels and later in public policy often determines the ongoing success of the vaccine initiative. This presentation will be part of the session “Identifying and Overcoming Communication Challenges.”

Methods

A review of vaccine initiative experiences in India, Japan and the United States focused on: 1) the identification of challenges and issues, 2) the response to them by sponsors or government partners, 3) community engagement with issues and solution identification, 4) the public health policy changes related to HPV vaccination.

Results

While top-down government support is important for success, community support is just as critical and can often be missed by program planners and policy partners at the mobilization stage. Various constructions of stakeholders and community partners exist, and do not necessarily involve individuals and groups who often experience the problems before planners might. A huge opportunity is risk communication planning, which must happen at the outset of planning and involve partners from across the spectrum of participation. Policies attempting to mitigate emerging issues are often initiated because issues were not addressed well initially.

Conclusion

Countries considering HPV vaccination campaigns or scale up are encouraged to engage not only implementation partners, but members of the community and especially groups that are wary of the effort.
Background / Objectives

HIV infection increases the risk for HPV-related cancers. This excess risk is due to a combination of increased sexual exposure to HPV infection and the influence of HIV-related immunodeficiency, as measured by CD4+ counts, on HPV natural history. Lower CD4+ counts have been shown to be associated with: 1) increased prevalence and persistence of cervical HPV infection, as well as the incidence of CIN2/3 and cervical cancer, 2) anal cancer incidence.

Methods

In order to estimate the burden of HPV-related cancer in persons infected with HIV (PHIV), we calculated incidence rates from record linkage between HIV/AIDS registries and cancer registries. Rates were applied to estimates of the population of PHIV in the United States in 2008 to obtain the total cancer burden. Site-specific attributable fractions and corresponding 95% confidence intervals (CIs) were estimated from infection prevalence among cancer cases derived from literature review of case series.

Results

Of an estimated 6,200 incident cancer cases diagnosed among ~800,000 PHIV living in the USA in 2008, 632 cases (10% of all cancers) were attributed to HPV. A majority of these HPV-related cancers were anal cancers (n=425). Cervical, vulva, vagina, penis and oropharynx cancer accounted for 86, 39, 2, 9 and 70 cases respectively. Incidence rates of HPV-related cancer increased after age 30 in PHIV. MSM were the HIV transmission group with the highest HPV-related cancer burden, among whom anal incidence exceeds 100 per 100,000 person-years in the era of combined antiretroviral therapy (cART). In female PHIV living in less developed settings with no cervical screening, the burden of cervical cancer is much higher than that estimated for the USA PHIV population.

Conclusion

The burden of HPV-related cancer in the HIV-positive population is high but also amendable to prevention, namely via 1) early detection and treatment of HIV infection to avoid immunosuppression, 2) universal HPV vaccination, and 3) for cervical cancer in particular, detection and treatment of precancerous lesions.
Background / Objectives

Women living with human immunodeficiency virus (WLHIV) are at elevated risk of cervical cancer and precancer. While screening guidelines have changes for women in the general population, optimal screening among WLHIV is less clear.

Conclusion

We will review data using a risk benchmarking approach to compare cervical precancer risks among WLHIV to general population risks that current U.S. guidelines are based on. The analysis identified groups of WLHIV that have low bHSIL+ risks (<1% at 3 years), and others that had much higher risk. How these data compare to and inform screening guidelines is discussed.
Strong T cell responses after vaccination with HPV16 long peptides for late stage cervical cancer are associated with prolonged survival


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Background / Objectives

Therapeutic vaccination with HPV type 16 synthetic long peptides (HPV16-SLP) results in T cell–mediated regression of HPV16-induced premalignant lesions but fails to install effective immunity in patients with advanced HPV16-positive cervical cancer. We showed that HPV16-SLP vaccination in mice and in patients with advanced cervical cancer patients fosters robust HPV16-specific T cell responses, when combined with chemotherapy (Welters et al. Sci. Transl. Med., 2016).

Methods

We have now completed a chemo-immunotherapy study in 70 patients with late stage HPV16+ cervical cancer (clinical trials.gov NCT02128126). Three HPV16-SLP vaccine doses were given 2 weeks after the second, third and fourth cycle of standard chemotherapy (carboplatin, AUC 6; paclitaxel 175 mg/ m2). Cohorts of 12 patients each were vaccinated with each of 4 dose levels (20, 40, 100 and 300 µg/ per peptide) of 13 overlapping HPV16 synthetic long peptides (HPV16-SLP) together covering the length of the 2 E6 and E7 proteins. Two additional cohorts of 6 patients each were vaccinated with the most promising doses of 40 and 100 µg/ peptide.

Results

Robust vaccine-induced HPV16-specific T cell responses as assessed by interferon-γ Elispot were observed and were sustained until at least 30 days after the 6th cycle of chemotherapy. In addition the chemotherapy augmented recall responses to microbial antigens. Such robust T cell responses were not noted in previous trials when similar patients were vaccinated without timing of vaccination during
chemotherapy. A marked and significant positive correlation was observed between the strength of the vaccine-induced immune response and overall survival. No such correlation was observed between the strength of the T cell response against common recall antigens and survival.

In addition a remarkably high proportion of patients survived beyond 2 years after the start of therapy.

Conclusion

The results suggest that survival duration is directly related to the strength of the vaccine-induced HPV16-specific T cell response and is not due to generally better immuno-competence.
EFFICACY OF A CARRAGEEANAN-BASED LUBRICANT GEL AGAINST HPV INFECTION IN WOMEN: INTERIM ANALYSIS OF A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Background / Objectives

To evaluate the efficacy of a carrageenan-based lubricant gel in reducing the risk of genital HPV infections among young sexually active women.

Methods

Between January 2013 and December 2016, 258 women aged 18 years and older were randomly assigned (1:1) to a carrageenan or a placebo lubricant gel. Participants were asked to self-apply the gel every other day for the first month and prior to and following each intercourse during the entire 1-year study period. Sociodemographic, behavioral and sexual history data were collected using computer-assisted self-administered questionnaires. We used Roche’s Linear Array assay to detect and genotype HPV DNA in self-collected vaginal samples. HPV types were categorized according to phylogenetic grouping based on tissue tropism and oncogenicity (alphapapillomavirus subgenus 1, 2 and 3). The primary outcome was the incidence of a newly detected infection i.e. an infection by an HPV type that was not present at baseline. We computed hazard ratios (HR) and 95% confidence intervals (CI) using univariate Cox models considering the first occurrence of a new infection in each participant. We also used a mixed effect survival-time model to accommodate the correlated data structure and multiplicity of HPV types to which participants were exposed.

Results

The median age was 22.7 years (range 18.0-45.2) and the median follow-up time was 7.6 months (range: 0-25.3) completed over up to 7 visits (mean: 4.8, median: 5). Baseline characteristics including age, ethnicity, age at first intercourse, number of lifetime sex partners, number of partners in the last month, HPV vaccination status and HPV status were well balanced between arms. 40 participants in the carrageenan arm and 59 participants in the placebo arm got infected by at least one new HPV type. The HR for the first occurrence of a new infection was 0.59 (95% CI:
A lower incidence was consistently observed for all alphapapillomavirus subgenera (HR range: 0.44-0.61). When considering all new HPV types acquired by each participant (not only the first infection), 89 infections occurred in the carrageenan arm versus 152, in the placebo arm. The HR was 0.45 (95% CI: 0.27-0.76) taking into account correlated data.

**Conclusion**

Although preliminary and based on interim analysis, our trial suggests that the use of a carrageenan-based lubricant gel can reduce the risk of new genital HPV infections, irrespective of taxonomic grouping.
DEMETHYLATING TREATMENT INDUCES A DOSE- AND TIME-DEPENDENT REVERSAL OF THE MALIGNANT PHENOTYPE AND ANTI-PROLIFERATIVE EFFECTS IN TWO- AND THREE-DIMENSIONAL HPV TUMOR MODELS

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Background / Objectives

Hypermethylation of both the viral and host genome is a frequent event during HPV-induced carcinogenesis. Specifically, methylation in the binding sites of the HPV E2 protein (E2BS) inducing HPV E6/E7 oncogene overexpression and of tumor suppressor gene promoters have prompted the concept that demethylating agents could represent a novel therapeutic approach against HPV-induced (pre-)cancerous lesions. Previous experiments in our department demonstrated a dose-dependent reversal of the malignant phenotype upon short-term treatment of HPV-transformed cells with the demethylating agent 5-aza-2'-deoxycytidine (DAC). We sought to analyze DAC-induced effects on HPV-transformed cervical cells in greater detail applying long-term DAC treatment in 2-dimensional cell culture. Further, we analyzed consequences of long-term DAC treatment on cell proliferation and tumor cell invasiveness in 3-dimensional tumor models of HPV-transformed cells.

Methods

HPV-transformed cell lines (CaSki, SiHa) were treated over a period of one and two weeks with different concentrations (0.1, 0.5, and 1.0 µM) of the demethylating agent DAC. Expression of the viral oncogenes was determined by qPCR (E6*I, E7) and Western Blot (E7). p16\(^{\text{INK4a}}\) and p53 protein levels were analyzed by Western Blot. β-galactosidase staining was performed to determine the induction of cellular senescence in treated cells. Tumor spheroids of CaSki and SiHa cells were established, treated with different concentrations of DAC over a period of one and two weeks, respectively, and cellular proliferative activity was analyzed using EdU immunofluorescence. A three-dimensional organotypic culture (OTC) model of CaSki cells was established, treated with DAC and analyzed for proliferative activity and tumor cell invasiveness applying p16\(^{\text{INK4a}}\)/Ki-67 immunohistochemistry (IHC).

Results

We observed a dose-dependent significant reduction of cellularity and reversal of the malignant cell phenotype characterized by significant down-regulation of viral
oncogene and p16\textsuperscript{INK4a} expression, up-regulation of p53 protein levels and induction of cellular senescence measured by β-galactosidase staining in treated CaSki and SiHa cells. All effects were even further pronounced in the second week of treatment. We could further demonstrate anti-proliferative effects of DAC treatment in 3D-models, indicated by a dose- and time-dependent reduction of EdU staining in CaSki and SiHa tumor spheroids and reduced p16\textsuperscript{INK4a} and Ki-67 expression in the CaSki OTC model, which was accompanied by a significant reduction of tumor cell invasiveness.

**Conclusion**

Demethylating treatment represents a promising novel therapeutic strategy for HPV-transformed (pre-)cancerous lesions.
SS 04-05
CRISPR/Cas9 Treatments to Eliminate HPV and other Persistent Viral Infections

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Background / Objectives

A latent virus can remain in a host indefinitely, causing persistent infections. Because the viral genome is not fully eradicated by the host’s immune system, the virus may reactivate periodically. More serious ramifications of latent viral infection include the possibility of transforming the cell or forcing the cell into uncontrolled division, causing various types of cancer. While antiviral therapies can suppress active viral replication, no existing treatment can effectively eradicate latent infection. During latent infection, the dormant viral genome provides few therapeutic targets other than itself for antiviral drug development, and therefore a cure is lacking for many viral diseases of critical unmet medical need. We have developed an RNA-based CRISPR/Cas9 antiviral platform to disrupt intracellular viral DNA while leaving the host genome untouched for the treatment and elimination of persistent viral reservoirs.

Methods

Our first product is designed to disrupt human papillomavirus 16 (HPV 16) viral genome in infected cells. This product is designed for local topical application to the mucosal non-keratinized epithelium where the basal epithelial cells harbor the persistent HPV infection and is intended for patients with high-grade squamous intraepithelial lesions (HSIL), a precancerous condition where surgical excision is the current standard of care and where recurrence rates are significant.

Results

Our HPV-CRISP/Cas9 antiviral has been well characterized using pharmacologically relevant cell models, along with rodent studies, to support in vivo target site uptake and RNA delivery. The product design was optimized to maximize the disruption of the HPV 16 genome with very high specificity (>99.9% as assessed by GUIDE-seq). We are currently advancing through preclinical development and are aiming to initiate clinical evaluation next year.

Conclusion

This CRISPR/Cas9 anti-HPV product will expand current HPV treatment options. By specifically targeting the HPV DNA that can hide from the immune system and from small molecule antivirals, this topical product is being designed to eliminate the viral reservoir and avoid recurrence and cancer progression. In addition to HPV, this
platform is being advanced to target other persistent viral infections, including HBV, EBV and cytomegalovirus (CMV).

References

Background / Objectives

Background: Oropharyngeal HNSCCa is frequently associated with HPV. We hypothesize that immunotherapy with INO-3112 will generate immune responses in patients (pts) with HPV+ HNSCCa.

Methods

Methods: This Phase I/IIa trial included pts with p16+ locally advanced HNSCCa, ECOG PS 0-1. INO-3112 was delivered IM along with electroporation using the CELLECTRA® device, every 3 weeks x 4 doses. Cohort 1 (C1) pts received INO-3112 pre and post-surgery; Cohort 2 (C2) pts received INO-3112 post cisplatin-based definitive chemoradiation. Primary and secondary endpoints were safety and immunogenicity. Pre- and post INO-3112 tissue samples (C1) were assessed for tumor infiltrating lymphocytes (TILs) using immunohistochemistry for CD8, FoxP3, PD-L1 and Granzyme B. Peripheral immune responses were assessed by ELISA to measure HPV16/18 specific antibody levels, and by IFNγ ELISpot to measure HPV16/18 specific T cell magnitudes at each dosing visit and every 3 months (mos).

Results

Results: As of March 2017, 22 pts were treated, completing accrual. C1: n=6, C2: n=16; 20 male, median age 57.5 years (32-76); primary tumor location: base of tongue=10, tonsil=12; never smoker=10. All pts are alive, median follow up is 16.4 mos (1-26). INO-3112 was well-tolerated with no related Grade 3-5 adverse events. In 5 C1 pts post immunotherapy, an increase in CD8+ infiltration into tumor was noted in 2 pts (1.6-3.6 fold) and a decrease in FoxP3+ was noted in the other 3 (1.8-2.1 fold). This resulted in positive shift in CD8+/FoxP3+ ratio in neoplastic tissue in 4/5 pts. Furthermore, increased PD-L1 and granzyme B expression was noted in 3/5 subjects (one PD-L1 alone, one Granzyme B alone and one for both PD-L1 and Granzyme B). Peak mean/median antibody responses to HPV16 E7 and HPV18 E7 antigens for 19 evaluable pts were 1:1235/1:150 and 1:2853/1:450, respectively. As compared to baseline, 18 evaluable pts showed elevated HPV16/HPV18 specific T cell activity (by IFNγ ELISpot), with peak mean/median responses of 179.99/68.33
SFU per $10^6$ PBMC (HPV16) and $107.18/53.3$ SFU per $10^6$ PBMC (HPV18). Persistent cellular responses $>100$ SFU/$10^6$ PBMC were noted out to 12 mos. 3 out of 22 pts have progressed; 1 pt received Nivolumab for progressive disease, and remains in complete response.

**Conclusion**

Conclusion: These data show that INO-3112 generates HPV-specific peripheral humoral and cellular immune responses that may persist out to 12 months and influences the composition of CD8+ and FoxP3+ immune infiltration into tumor tissue in HPV+ HNSCCa.
DEVELOPMENT OF A THERAPEUTIC CANCER VACCINE BASED ON p16INK4a

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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 p16INK4a 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.

p16INK4a expression has also been detected in non-HPV-related tumor entities as colorectal and small cell lung cancer, suggesting p16INK4a as a broad tumor associated antigen that is not only specific for HPV-associated cancers.

In a phase I/IIa trial to monitor toxicity and immunogenicity of a p16INK4a peptide vaccine in patients with advanced HPV-associated, p16INK4a-overexpressing cancers, we could show the induction of a humoral and cellular immune response against p16INK4a without any severe vaccine-related side effects.

Presently we are establishing a p16INK4a-positive tumor mouse model in order to explore the effect of a p16INK4a-based vaccine on tumor growth and its potential to be combined with current immunotherapies.

Methods

Pools of long, synthetic peptides covering the whole p16INK4a sequences were injected into C57BL/6 mice. Establishing the p16INK4a-positive tumor mouse model, C57BL/6 mice were challenged with p16INK4a-expressing TC-1 cells before and respectively after the peptide immunizations to analyse the tumor response of a therapeutic and respectively prophylactic vaccine approach.

Results

The p16INK4a-based vaccination with long, synthetic p16INK4a peptides induced an antibody response against p16INK4a detected by ELISA as well as IFNγ-producing T
cells measured by ELISpot. We are currently performing the tumor regression and protection experiments with p16\textsuperscript{INK4a}-positive TC-1 tumor cells in C57BL/6 mice.

**Conclusion**

The established murine system allows us to address the question whether a p16\textsuperscript{INK4a}-based vaccine is able to induce the regression of an established p16\textsuperscript{INK4a}-positive tumor and/or to prevent the further outgrowth of a tumor expressing p16\textsuperscript{INK4a}.

The generation an effective tumor response against p16\textsuperscript{INK4a} could lead to a new therapeutic approach for HPV-induced cancers as well as for tumors overexpressing p16\textsuperscript{INK4a} independent of an HPV infection.
Background / Objectives

Persistent infection with high-risk (HR) human papilloma virus (HPV) is a necessary condition for cervical cancer (CC) development. Recently, several scientific reports indicated the role of vaginal microbiota in the acquisition and persistence of HPV and subsequent development of CC (1). In order to identify metagenomic markers predictive of HR-HPV persistence, we characterized the vaginal microbiota from women screened for HR-HPV comparing microbiota profiles to HR-HPV status (clearance or persistence), after one year follow-up.

Methods

Seventy cervico-vaginal HPV –HR positive samples were obtained from a biobank collected within HPV DNA based primary screening program (ISPO, Florence, Italy). The absence of cervical grade lesions was ascertained by colposcopy. Follow-up results after one year from the first sampling were used to further divide the group of HR-HPV+ donors in (i) Clearance: HR-HPV+, including n=27 samples from patients with no evidence of HR-HPV DNA after one year; (ii) Persistence: including n=28 HR-HPV+ samples from patients that maintained the expression of HR-HPV-DNA in the cervico-vaginal environment after one year from the first screening. Pyrosequencing of V3-V5 region of 16S-rDNA gene was performed on bacterial genomic DNA purified from cervicovaginal samples and was used to characterize microbiota and to define community state types (CSTs) in each sample. Enriched taxa were identified by Linear discriminant analysis effect size (LEfSe). Expression of Gardnerella vaginalis sialidase gene was studied by PCR.

Results

Metataxonomic analysis showed differential microbiota profiles between HPV- and HPV+; an increased biodiversity was revealed in the group of persistence compared to the group of clearance and to the control group. A CST IV subgroup, dominated by selected anaerobic genera (Gardnerella, Prevotella, Megaspheora, Atopobium), frequently associated with bacterial vaginosis (BV), was present in 43% of women in the group of persistence and in only 7% of patients in the group of Clearance. Samples from patients developing persistent HPV infection showed
significant enrichment in Atopobium, as well as a high frequency of Gardnerella strains producing sialidase.

Conclusion

The observed differential cervico-vaginal microbiota profiles in women with HPV infection suggest important insight on the role of bacterial vaginal microbiota in HPV infection. We propose the CST IV (BV) subgroup as a risk factor for the persistence of HPV infection and Atopobium as microbial markers of viral persistence.

References

SS 05-01
THE STATE OF THE ART OF HPV ASSOCIATION IN NON-GENITAL, NON-ORAL CANCERS. AN OVERVIEW.

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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 recent 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 pooled prevalence 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; pooled prevalence of 33.0% (95%CI, 24.9-42.3%; RE model). Seventy-six studies were eligible covering 1,956 sinonasal papillomas from all geographic regions. Altogether, 760 (38.8%) cases tested HPV-positive; effect size 42.1% (95%CI 35.9-48.5%, RE model). Of laryngeal SCCs, 180 studies were eligible comprising a sample size of 7,353 laryngeal SCCs. Of these, 1,833 (25%) tested HPV-positive (any methods); effect size 26.9% (95%CI 24.2-29.7%; RE model). Exactly 100 studies on lung cancer were eligible, covering 7,381 lung cancers (all histological types). In total, 1,653 (22.4%) samples tested HPV-positive; effect size was 22.0% (95% CI=18.0-25.9%; RE model). Of the 1,177 abstracts found on ESCC, 152 studies were consider eligible. These 152 studies covered 10,234 ESCC cases, analysed by different HPV detection methods. Altogether, 3,135 (30.6%) tested HPV-positive, translating to an effect size of 29.0% (95%CI 25.1-31.0; RE model).

Conclusion

The results of these published meta-analysis indicate that the reported wide variability in HPV detection in SNC, LC, BC and EC is not due to the HPV detection techniques, but best explained by the geographic origin of the study, except in SNC. In LC, also the histological type is a significant study-level covariate.
References


Background / Objectives

Laryngeal squamous cell carcinoma (LSCC) represents the second most common malignancy in the head and neck worldwide. Traditionally, tobacco and alcohol exposure are the main risk factors for LSCC, however, molecular evidence has linked HPV, particularly HPV-16, in the pathogenesis of a subgroup of LSCC cases. The rate of HPV detection among LSCC samples has remained highly variable, ranging from 0% to 85%. This large variability is commonly related to: 1) differences in geographic region and ethnic group; 2) inadequate distinction of patients with LSCC from those with other cancers of the head and neck region such as oropharyngeal SCC; and 3) differences in sensitivity and specificity of HPV genotyping methods and diagnostic criteria.

Methods

A meta-analysis was recently conducted including 179 articles published between January 1964 and March 2015 reporting HPV detection in LSCC.

Results

A sample size of 7,347 LSCCs was evaluated and the observed HPV prevalence was 25%. A variability in the prevalence of HPV in LSCC was observed according to 1) HPV detection method and 2) geographic origin. However, only the variability related to geographic area of the study reached statistical significance in stratified meta-analysis and when formal meta-regression was conducted, none of these characteristics were significant study-level covariates accounting for the heterogeneity of HPV prevalence. Noteworthy, the mere presence of HPV DNA in a tumor does not necessarily indicate that the virus is driving or contributing to tumor development or progression of LSCC. Although HR HPV can be found in LSCC, when using a rigorous definition of truly transcriptionally-active HPV-related, the rates are generally very low.
Conclusion

Despite the remarkable heterogeneity between methods for HPV status testing across the studies, variability on detection rates in LSCC seemed to be only explained by geographic origin, maybe reflecting genetic diversity or environmental and cultural differences. Noteworthy, these studies did not analyze the physical state or transcriptional activity of HPV in these tumors. Therefore, multicentric follow-up studies and functional studies are needed to further characterize the role of HPV in LSCC.
ESOPHAGEAL CARCINOMA: ANY ROLE FOR HPV?

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Background / Objectives

Esophageal cancer is the eighth most common cancer, which shows remarkable regional variations in incidence rates. The etiology of the most common type of esophageal cancer - esophageal squamous cell carcinoma (ESCC), is undisputedly multifactorial and includes the synergistic action of Group 1 human carcinogens: tobacco, betel quid with or without added tobacco, alcohol and acetaldehyde associated with alcoholic beverages. According to the robust and strong data, which have accumulated over last 30 years, carcinogenic HPV types from the α-PV genus, mainly HPV-16, should also be seriously considered as etiological agents for a subset of ESCC.

Methods

Recent meta-analyses and our literature update identified more than 200 studies with over 13,000 cases of ESCC analyzed for the presence of HPV to date. Approximately one third of ESCC specimens tested HPV positive, but HPV DNA detection rates were highly variable in different geographical areas of the world. A meta-analysis of case-control studies investigating the etiological role of HPV in the development of ESCC also supports an HPV-ESCC association for fraction of ESCC. Our literature review showed that the two most important vaccine-preventable HPV types: HPV-16 and HPV-18 have been the most commonly identified HPV types in ESCC specimens in both low-incidence and high-incidence settings. Prophylactic vaccination with all three currently available HPV vaccines could theoretically prevent more that 70% of all alpha-HPV DNA positive ESCC, although only if proved to work against non-genital HPV-related cancers or their precursors.

Conclusion

In order to provide conclusive evidence that HPV is a definitive causative factor in subset of ESCCs, we need more studies. A well-designed large international case-control study with sufficient power indisputably to ascertain HPV rates in ESCC cases compared to controls without ESCC using a uniform HPV testing methodology is the most practical way forward.
Active human papillomavirus involvement in Barrett’s dysplasia and oesophageal adenocarcinoma is characterized by wild-type p53 and aberrations of the retinoblastoma protein pathway


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Background / Objectives

We have previously demonstrated that transcriptionally active high-risk HPV (hr-HPV) is strongly incriminated in Barrett’s dysplasia (BD) and oesophageal adenocarcinoma (OAC) using mainly fresh frozen tissue.1-4 This study aimed to identify biomarkers of active HPV infection in Barrett’s metaplasia, (BM)/BD/OAC by immunohistochemical staining (IHC) of formalin-fixed paraffin embedded (FFPE) tissue for aberrations of p53 and the retinoblastoma (pRb) pathway which are targets for the viral oncoproteins, E6/E7 respectively.

Methods

Prospectively, BM(n=81)/BD(n=72)/OAC(n=65) FFPE specimens were subjected to IHC staining for pRb, p16INK4A, cyclin D1, p53 and RNA in-situ hybridization (ISH) for E6/E7 transcripts. HPV DNA was determined via PCR in fresh frozen specimens. Viral load measurement (real-time PCR) and Next Generation Sequencing of TP53 was also performed.

Results

Of 218 patients, 56 were HPV DNA positive [HPV16 (n=42), 18 (n=13), 6 (n=1)]. Viral load was low. Transcriptionally active HPV (DNA+/RNA+) was only found in the dysplastic and adenocarcinoma group (n=21). The majority of HPV DNA+/RNA+ BD/OAC were characterized by p16INK4Ahigh (14/21, 66.7%), pRblow (15/21, 71.4%) and p53low (20/21, 95%) and was significantly different to controls [combination of HPV DNA-/RNA- (n=94) and HPV DNA+/RNA- cohorts (n=22)] p53low had the strongest association with DNA+/RNA+ oesophageal lesions (OR=23.5, 95% CI=2.94-187.8, p=0.0029). Seventeen HPV DNA+/RNA+ BD/OAC identified as p53low, were sequenced and all but one exhibited wild-type status. pRblow/p53low provided the best balance of strength of association (OR=8.0, 95% CI=2.6-25.0, p=0.0003) and sensitivity (71.4%)/specificity (71.6%) for DNA+/RNA+ BD/OAC.
Conclusion

Active HPV involvement in BD/OAC is characterized by wild-type p53 and aberrations of the retinoblastoma protein pathway.

References


SS 05-05
HPV TRANSCRIPTION IN NON-MELANOMA SKIN CANCER AND CERVICAL CANCER

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Background / Objectives

The increased incidence of non-melanoma skin cancer (NMSC) among immunosuppressed patients has suggested that infectious agent(s) may be involved. In previous studies, sequencing of skin lesions identified >400 different HPVs, including over 200 putatively novel types. Cervical cancer is known to be caused by HPV; however, some tumors appear to be HPV-negative by primer-based detection systems. As all established oncogenic infectious agents persist transcriptionally active in their host, we wanted to analyze tumors by RNA-sequencing to investigate if there is transcriptionally active HPV present.

Methods

NMSC biopsies from 2 patients, cervical cancer biopsies from 6 patients (including 4 apparently “HPV-negative” by primer-based detection) together with an HPV-positive cervical cancer cell line control (CaSki cell-line, known to contain around 600 copies of integrated HPV 16 DNA) were analyzed by RNA-sequencing.

Results

RNA from HPV type 110 was detected in the 2 NMSC tumors (106 and 10 reads respectively). RNA from one or more HPV types (HPV types 119, 128, 129, 144, 147 and 16) were detected in 5 of 6 cervical cancer samples (including 3 “HPV-negative” cancers). HPV type 16 was also detected in the HPV-positive cell line control (18386 reads). The results implicate that the HPV genomes in the NMSC are actively transcribed. The transcribed HPV genes detected in the NMSC tumor with 106 reads were E6, E7, E1, E2/E4 and L2, whereas L1 was not detected. Only E7-transcription was detected in the NMSC tumor with 10 reads. Highly transcribed HPV genes in the CaSki cell line were E6, E7, L1 and L2, whereas E2, E4 and E5 showed very little transcription and E1 was not represented with a single read.

Conclusion

RNA metagenomic sequencing can be used to detect transcriptionally active HPV infection in NMSC and cervical cancer that have been “HPV-negative” by primer-based HPV-detection.
SS 05-06

DEVELOPMENT OF A PATIENT FRIENDLY SAMPLING METHOD FOR SKIN DISORDERS: CUTANEOUS WARTS AS A CASE-STUDY

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Background / Objectives

The involvement of HPV in development of mucosal disorders has been widely established. Specific cutaneous disorders have also been associated with HPV, however the exact role of HPV remains largely unknown. The lack of optimization and standardisation of the pre-analytical phase forms a major obstacle. The aim of this study was to develop an accurate/patient friendly sampling method for skin disorders, with cutaneous warts as a case study. An additional aim was to create an HPV qPCR genotyping assay capable of detecting the most prevalent wart-associated HPV types (i.e. HPV1, 2, 3, 4, 7, 10, 27, 41, 57, 60, 63 and 65).

Methods

Several sampling methods were examined, i.e. skin scrapings (n=5), swabs (n=6) and a tape-based sampling method (n=6). In addition, the performance of two different swabs, i.e. cotton (Abbott MC Specimen Collection) and flocked (FLOQSwab Copan Diagnostics) was analysed. In total 32 warts were sampled by both types of swabs in an alternating order. The optimized DNA extraction protocol involved overnight Proteinase K and EDTA digestion, followed by automated extraction on the NucliSENS® easyMAG® system (bioMérieux). Quantification of the DNA yield was achieved by B-globin qPCR (cell control) and Kruskal-Wallis and Paired Student T-test were used to compare Ct-values. A wart-associated HPV genotyping qPCR assay, able to detect the above mentioned cutaneous HPV types, was developed, containing type-specific primers and consensus probes capable of detecting multiple types.

Results

All samples tested positive for B-globin and were considered valid. Skin scrapings had significantly higher yield than both swab and tape-based methods (p<0.01), the latter two did not significantly differ from each other (p>0.05). When comparing B-
globin Ct values no significant difference was found between cotton and flocked swabs irrespective of sampling sequence (p >0.05). All swabs were HPV positive, however there were some discrepancies in HPV type-specific detection but these were not statistically significant and can be attributed to the assay detection limit (Pearson’s X2 test p>0.05; \( \kappa =0.79 \) [95%CI, 0.73-0.86]).

**Conclusion**

Although somewhat better DNA yield was found in skin scrapings, the patient discomfort was an important limitation of this method. Considering that, in combination with our optimized DNA extraction procedure, all samples gave valid results with the less invasive swab method this technique is preferred. An additional advantage of swabs is the option for automated pre-analytical processing, which is not feasible with the alternative methods. Performance of both types of swabs was demonstrated to be equal.
QHPV AND 9VHPV VACCINES: 20 YEARS OF CLINICAL RESEARCH & DEVELOPMENT

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Background / Objectives

The development of the 9vHPV vaccine represents the culmination of over 20 years of continuous HPV vaccine research & development. After the demonstration by several research groups that HPV L1 capsid protein expressed in a recombinant system forms VLPs, studies showed that VLPs can be disassembled and reassembled into more stable and immunogenic structures, which enabled commercial development. Proof-of-concept studies in animal models and the identification by IARC of HPV 16 and 18 as carcinogenic was followed by clinical development in 1997 using proof-of-principle studies of monovalent vaccines. The qHPV vaccine was evaluated beginning in 2000 as a 3-dose series in young women age 16-26 years and prevented HPV16/18-related cervical, vulvar, and vaginal high-grade dysplasia and HPV6/11-related genital warts. In 2004, efficacy studies of the qHPV vaccine administered as a 3-dose regimen were initiated in mid-adult women age 24-45 years and young men age 16-26 years; the qHPV vaccine prevented HPV6/11/16/18-related infection and cervical, vulvar, and vaginal dysplasia in mid-adult women; it also prevented HPV6/11/16/16-related anal dysplasia and genital warts in young men. Efficacy results of qHPV vaccine in young women and young men were extrapolated to pre- and young adolescent girls and boys based on the demonstration of non-inferior immunogenicity compared with women or men. It also became known in 2004 that HPV31/33/45/52/58 were the next most frequent types associated with cervical cancer; thus, a preliminary Phase II immunogenicity study was started in 2005 to identify a higher-valent HPV vaccine candidate. The 9vHPV vaccine was evaluated beginning in 2007 in over 14,000 young women age 16-26 years. Efficacy results in young women were extrapolated to girls, boys and young men based on the demonstration of non-inferior immunogenicity. A study of a 2-dose schedule in girls and boys age 9-14 years was initiated in 2013 and is expected to be completed in 2017. The bivalent HPV vaccine was developed in girls and women along similar timelines as the qHPV vaccine. The qHPV, bivalent HPV, and 9vHPV vaccines were first licensed in 2006, 2007 and 2014, respectively.

Methods

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Results

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Conclusion
LONG-TERM EFFECTIVENESS AND IMMUNOGENICITY OF GARDASIL™ IN THE NORDIC COUNTRIES

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Background / Objectives

The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of a pivotal study (Protocol 015) to investigate the safety, immunogenicity, and effectiveness of GARDASIL™ on the incidence of HPV 16/18-related CIN2 or worse in 16-23-year old women. Here, we analyze the effectiveness and immunogenicity of the vaccine in this population of women up to 10-12 years after the start of vaccination.

Methods

All women in the trial are followed through different national registries (Denmark, Iceland, Norway and Sweden) for immunogenicity and effectiveness and safety data. Interim effectiveness and safety analyses started approximately 2 years following completion of Protocol 015 and have been occurring every 2 years thereafter. Cohort 1 included approximately 2,700 subjects who received GARDASIL™ at the start of Protocol 015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and GARDASIL™ prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN2 or worse (CIN2+) was estimated by comparing the observed incidence with the expected incidence of CIN2+ in an unvaccinated cohort using historical registry data. This is the last interim analysis before the completion of the study (with 14 years of follow-up of the subjects in Cohort 1.)

Results

In the analysis of effectiveness after the first 12 years, there were 2,084 subjects that contributed to the follow-up period out of a total of 2,650 eligible subjects in the per-protocol population in Cohort 1. No new cases of HPV 16/18-related CIN 2 or worse observed. There were also no cases of HPV 6/11/16/18-related CIN, vulvar cancer, and vaginal cancer observed.

Conclusion

GARDASIL™ shows continued protection in women through 10 years, with a trend towards 12 years of protection.
Background / Objectives

We describe the final 10-year data for the long-term follow-up (LTFU) study of the 4vHPV vaccine in pre-adolescents and adolescents.

Methods

In the base study (V501-018), 1661 sexually naïve males and females received 4vHPV vaccine (EVG; Early Vaccination Group, followed for 9.9 years) or placebo at day 1, months 2 and 6. Thereafter at month 30, the placebo group (CVG; Catch Up Vaccination Group, followed for 7.4 years) received 4vHPV vaccine using the same dosing schedule. Long term anti-HPV type 6, 11, 16 and 18 immune responses were assessed. Effectiveness was estimated by calculating the incidence rate of the primary endpoints (HPV6/11/16/18 related disease or persistent infection).

Results

For each of HPV types 6, 11 and 16, 89%-96% of subjects remained seropositive through 10-years post-vaccination. The preadolescents had 38%-65% higher GMTs at month 7 which remained 16%-42% higher at 10 years compared to the adolescents. No cases of HPV type 6, 11, 16 and 18-related disease were observed. Ten subjects had persistent infection of ≥6months duration with vaccine-type HPV (females: 0.3/100 person-years at risk (EVG & CVG); males: 0.6/100 person-years at risk (EVG) and 0.4/100 person-years at risk (CVG). Infection persisted for ≥12 months in 2 subjects. No new serious adverse events were reported through 10 years.

Conclusion

A 3- dose regimen of the 4vHPV vaccine was immunogenic, clinically effective, and generally well tolerated in pre-adolescents and adolescents during 10 years of follow-up. These long term findings support efforts to vaccinate this population against HPV prior to exposure.
LONG-TERM EFFECTIVENESS OF GARDASIL™ AMONG ADULT WOMEN IN COLOMBIA

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Background / Objectives

The Future III base study was a 48-month randomized, placebo controlled, multinational study evaluating the prophylactic administration of GARDASIL™ in 24-45 year old women. The long-term follow-up (LTFU) study was conducted in Colombia to observationally describe the safety of quadrivalent HPV vaccine (qHPV vaccine), its immunogenicity and effectiveness in preventing HPV 6-,11-,16-,18-related cervical intraepithelial neoplasia (CIN) or condyloma for up to 10 years. We present the final study data.

Methods

The LTFU study enrolled 685 of 805 Colombian subjects who received qHPV vaccine during the base study (early vaccination group, EVG). Study visits were conducted at Year 6, 8 and 10 and included history taking, pelvic exams with Pap tests, and biopsy of cervical/external genital lesions if present. Endpoint adjudication was performed by an independent panel. Immunogenicity was measured for each vaccine HPV type. Primary analyses were performed per-protocol.

Results

There was no case of HPV 6-,11-,16-,18-related CIN or condyloma in the EVG during the LTFU study. The cumulative incidence probabilities from Year 4 to Year 8 and Year 6 to Year 10 of the LTFU study were 0.0, respectively, compared to 0.0006 (95% confidence interval 0.0001; 0.0045) for the 4-year interval of the base study, indicating no waning of vaccine effectiveness. Vaccine induced HPV type-specific antibody responses were durable. No serious adverse events related to qHPV vaccine or study procedure were reported.

Conclusion

The prophylactic administration of qHPV vaccine to mid-adult women is effective and generally safe through 10 years post Dose 1 and induces durable immune responses.
A LONG-TERM EFFECTIVENESS, IMMUNOGENICITY, AND SAFETY STUDY OF GARDASIL™ (HUMAN PAPILLOMAVIRUS [TYPES 6,11,16,18] RECOMBINANT VACCINE) IN YOUNG MEN (V501-020)

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Background / Objectives

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.
Efficacy and Immunogenicity of the 9-Valent HPV Vaccine: Final Analyses of a Randomized, Double-Blind Trial with Up to 6 Years of Follow-Up

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Background / Objectives
The 9-valent human papillomavirus (9vHPV) vaccine targets the four HPV types (HPV6/11/16/18) covered by the quadrivalent HPV (qHPV) vaccine, with the addition of the five oncogenic types most commonly associated with cervical cancer after HPV16/18 (HPV31/33/45/52/58). Primary analyses of a study in young women 16-26 years of age demonstrated efficacy of 9vHPV vaccine against HPV31/33/45/52/58-related infections and disease, and non-inferior HPV6/11/16/18 antibody responses when compared with qHPV vaccine. This presentation evaluates study outcomes, including efficacy for up to 6 years following first administration and antibody responses. Additionally, vaccination impact on cervical cytology abnormalities and related therapeutic procedures are reported.

Methods
In this randomized, double-blind study (Protocol V503-001; NCT00543543), participants (N=14,215) received a three-dose series of 9vHPV or qHPV (control) vaccine. Cervical and external genital swabs for HPV-DNA testing, and cervical cytological samples for Papanicolaou staining were collected regularly. Tissue samples from biopsy or cervical definitive therapy (loop electrosurgical excision procedure, conization) were tested for HPV DNA. Serum antibody responses to the nine vaccine HPV types were assessed.

Results
In the pre-specified, per-protocol population (susceptible population), efficacy against HPV31/33/45/52/58-related cervical intraepithelial neoplasia Grade 3 (CIN 3) was 100% (95% CI: 39·4, 100). Efficacy against HPV31/33/45/52/58-related cervical, vulvar, and vaginal disease; persistent infection; cytological abnormalities; cervical biopsy; and cervical definitive therapy ranged from 90-98%. Incidence of HPV6/11/16/18-related persistent infection, disease, cytological abnormalities, and procedures was similar in 9vHPV and qHPV vaccine recipients. Antibodies to the HPV types targeted by each vaccine persisted through 5 years following vaccination. Geometric mean titer ratios (9vHPV/qHPV) for HPV6/11/16/18 remained stable over time. Administration of a fourth dose to a subset of participants 5 years after vaccination resulted in a rapid increase of GMTs for all 9 HPV types, which is indicative of immune memory.
Conclusion

The 9vHPV vaccine prevents >90% HPV31/33/45/52/58-related infection, cytological abnormalities, high-grade lesions, and cervical procedures in a prophylactic setting. Both vaccines protected against HPV6/11/16/18-related infection, cytological abnormalities, and high-grade disease and had a similar immunogenicity profile with respect to HPV6/11/16/18. Vaccine efficacy was sustained for up to 6 years.
SS 06-07

DESIGN OF A LONG-TERM FOLLOW-UP
EFFECTIVENESS, IMMUNOGENICITY AND SAFETY
STUDY OF WOMEN WHO RECEIVED THE 9-VALENT
HUMAN PAPILLOMAVIRUS VACCINE

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Background / Objectives

The efficacy of the 9-valent human papillomavirus vaccine (9vHPV) to prevent infection and disease was established in a Phase III clinical study in women 16-26 years of age. Here we present the unique design of a long-term follow-up (LTFU) study to assess effectiveness of the 9vHPV vaccine for up to 14 years post start of vaccination.

Methods

A long-term follow-up (LTFU) study was initiated as an extension of the Phase III clinical study to assess effectiveness of the 9vHPV vaccine for at least 14 years after the start of vaccination. It includes participants from Denmark, Norway and Sweden and uses national health registries from these countries to assess incidence of cervical precancers and cancers due to the 7 oncogenic types in the vaccine (HPV 16/18/31/33/45/52/58). Incidences will be compared to the estimated incidence rate in an unvaccinated cohort of similar age and risk level. This LTFU study is unique in design as it is an extension of a Phase III clinical study and also has elements of an epidemiological study (i.e., endpoints based on standard clinical practice; surveillance using searches from health registries). A control chart method to determine whether vaccine effectiveness may be waning is utilized.

Results

Experience from this innovative study design using Control chart methods may be applicable to other medicinal products when long-term outcomes need to be assessed.

Conclusion

These methods can be used to monitor disease incidence in real-time and promptly detect a decrease in vaccine effectiveness.
SS 06-09
COMPARISON OF IMMUNOGENICITY OF 2-DOSE AND 3-DOSE REGIMENS OF 9-VALENT (9v)HPV VACCINE

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Background / Objectives

To compare HPV-antibody responses in girls and boys aged 9-14 years given 2 doses of the 9vHPV vaccine versus women aged 16-26 years given 3 doses.

Methods

Protocol V503-010 is an international immunogenicity study of the 9vHPV vaccine using 5 cohorts: (1) girls receiving 2 doses 6-months apart; (2) boys receiving 2 doses 6-months apart; (3) girls and boys receiving 2 doses 12-months apart; (4-control): young women receiving 3 doses (D1, M2, M6); (5-exploratory) girls receiving 3 doses (D1, M2, M6). HPV-type specific geometric mean titers (GMTs) were assessed 1 month post-last dose by competitive Luminex immunoassay. Primary objectives were to demonstrate non-inferior HPV GMTs 1 month post-last dose in cohorts 1, 2, and 3 compared to cohort 4. Statistical criterion for non-inferiority required the lower bound of the 95% confidence interval of GMT ratios (cohort 1, 2, or 3/cohort 4) each be >0.67 for all HPV types.

Results

GMTs for all 9 HPV types were non-inferior in girls and boys aged 9-14 years given 2 doses versus women aged 16-26 years given 3 doses (cohort 4). The primary objectives of non-inferior immunogenicity of 2-doses versus 3-doses were met. Most subjects seroconverted to all vaccine types. In girls aged 9-14 years, 2 doses elicited lower GMTs than 3 doses for some vaccine types.

Conclusion

Using these results, efficacy findings in young women given 3 doses of 9vHPV vaccine can be extrapolated to girls and boys 9-14 years old given 2 doses at 0, 6 or 0, 12 months.
SS 07-02
Implementation of HPV screening of cervical swaps and self-sampling – verification: design and results

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Background / Objectives

The renewed population screening for cervical cancer in the Netherlands started in January 2017. Two major changes compared to the program until then, were the switch towards primary HPV screening and the introduction of a self-sampling device for non-responders. Implementing changes within an existing screening program requires careful preparation. Therefore, extensive verification studies were carried out during implementation of HPV testing and use of self sampling.

Methods

Our goal was to verify that the Cobas 4800 HPV test in the Dutch screening laboratories performed similar to the clinical validation; furthermore for verification of the self-sampling we wanted to establish the optimal method of sample preparation and its feasibility in the routine lab flow. Cervical swaps were collected in PreservCyt, whereas self sampling was performed using the Evalyn brush. For HPV testing the Cobas 4800 HPV test platform was implemented, making use of the p480 de- and recapping, x480 sample processing and PCR setup, and z480 PCR amplification. The desing of the verification was written out in test protocols.

Results

Verification of HPV test: An initial test on reproducibility, precision and accuracy was carried out at the vendor’s site on a single system using a specific verification panel and subsequently repeated on all Cobas 4800 assay systems (n=15) in the 5 screening laboratories. In order to secure the technical performance of the equipment, an operational test was conducted, including two weeks fully operational running on high volume prior to the release of the systems. The HPV verification tests were successfully performed and the data fulfilled the criteria of reproducibility, precision and accuracy. All assay systems in the screening laboratories also met the functional requirements.

Verification of the self sampling: Statistical analysis of data from a previous study was used to determine the adequate liquid volume of sample processing: it appeared that processing the Evalyn brushes in standard 20 ml PreservCyt vials was sufficient and resulted perfectly in a uniform laboratory work flow for HPV testing using both
cervical scrapes and self sampling. In addition, a stress test was conducted to examine whether vials including brushes could safely be mechanically processed by the HPV assay systems.

Conclusion

Verification has proven to be useful in introducing two major changes within the cervical cancer screening in the Netherlands. Based upon the verification results, the Cobas 4800 systems were released for use within the renewed screening program and a standard operation procedure for the processing of self-sampling devices has been established and implemented.
Background / Objectives

Cervical cytology is a subjective assessment of cellular changes that could be biased by knowledge of an HPV status. With the advent of primary HPV screening in the Netherlands, the place of cytology will shift from a primary screening role to a secondary triage role. This may not only increase the percentage of abnormal cytology but this can be further enhanced by the knowledge of positive HPV status. We have set up this study to measure the possible effect of knowledge of the HPV status on cervical cytology as can be observed in the new primary HPV screening in the Netherlands.

Methods

Prior to the start of the renewed Dutch cervical screening program based on primary HPV testing, we have asked two pairs, each consisting of two experienced Dutch cytologists to examine and classify a set of 100 slides in two rounds, with a wash-out period of at least two months. About 50% of the slides was hrHPV positive. Ideally, this experiment would be conducted in a blinded manner in the five screening laboratories, in order to quantify any possible shift in classification of abnormality (expected to increase by 10-15%). However, this was considered too burdensome for the laboratories. Therefore, the experiment was done with two pairs of volunteers, providing them with limited information, i.e. a measurement in cytology reproducibility. Briefly, a set of glass slides was derived from a blinded pilot of hrHPV cotesting in population based screening in the Netherlands (DuSC study), adjusted for age and expected percentage of abnormalities and cleared of any marker on the slides. During the first round, slides were offered to the experts without any information on hrHPV status, while, after a two-month wash-out period, the reordered slides were offered with information on hrHPV status per slide. Each expert wrote down classification scores during each round. The interrater reliability and the test-retest reliability of the cytologists were calculated by means of Cohen's Kappa and evaluated.

Results
Results will be shown.

**Conclusion**

Based on preliminary results, the HPV-bias seems to be limited i.e. below the anticipated 15%. Abnormality rate of cytology should be measured carefully during the implementation of the program with adjustment of cytology criteria if necessary to avoid undue burdening of colposcopy clinics.
MONITORING AND FIRST RESULTS OF THE RENEWED HRHPV BASED POPULATION SCREENING FOR CERVICAL CANCER IN THE NETHERLANDS

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Background / Objectives

In January 2017 the renewed cervical cancer screening programme based on primary hrHPV testing has been implemented in the Netherlands. For quality assurance - to ensure an optimal balance between the benefits and harms of screening - it is important to closely monitor the screening programme. Regular monitoring by calculation of screening indicators is required both to identify and solve specific bottlenecks that are impeding an efficient screening programme, and more generally to ensure that the public finances continually invested in the programme are spent in a responsible way. We calculated such a set of short-term screening process indicators, that are available early in the lifetime of the screening programme, to observe the effects of the renewed screening programme in the Dutch population.

Methods

We calculated the attendance rate (divided by regular screening and self-sampling) and the proportion of HPV positive tests by using data from the screening organisations. Information on all cytological and histological examinations of the cervix uteri taken in the Netherlands from January 2017 onwards were available and retrieved from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). By using these data, we calculated the proportion of cytological abnormalities, the detection rates of CIN and cervical cancer. All indicators are calculated by different age groups.

Conclusion

The first results of the new hrHPV-based screening programme in the Netherlands look promising. The number of HPV positive women is high, but not higher than expected. It is important to keep monitoring the effects of the screening programme, especially the number of women participating in the self-sampling, the rate of opportunistic screening and the number of women send to colposcopy, to ensure the
quality of the programme. In the long term other screening indicators are important, such as the interval cancer rate.
How and when to screen a vaccinated cohort for the first time

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Background / Objectives

Many vaccinated women have reached the age of first screen. Screening registers have shown that the incidence of CIN2 or worse (CIN2+) decreases substantially as a consequence of vaccination and therefore, countries need to rethink the age of the first screen as well as the primary screening instrument. Data sources that can inform screening strategies for vaccinated women include randomized screening trials performed in vaccinated women, and screening cohorts and registries in which the occurrence of prevalent and incident genotype-specific HPV infections and CIN are estimated.

Methods

An overview of the evidence in the literature will be given. Furthermore, the Dutch POBASCAM and VUSAScreen trial will be used to illustrate how vaccines with protection against HPV 16/18 and 16/18/31/33/45/52/56 are expected to influence the HPV prevalence and the positive predictive value for detection of CIN3+ at the first screen. The impact of vaccination on CIN3+ will be calculated under the assumption of a hierarchical and proportional classification of type-specific CIN lesions, and by means of a statistical approach that assumes that type-specific CIN3+ risks are independent.

Conclusion

An important measure for advising on the age of first screen is the positive predictive value for detection of CIN3+. The positive predictive value may decrease substantially when vaccinated cohorts enter screening which supports an increase of the starting age of screening.
Screening options and challenges in women immunised with bivalent or quadrivalent vaccines

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Background / Objectives

HPV vaccination is having a profound influence on HPV infection and associated disease in countries that have adopted national immunisation programmes. In Scotland -90% uptake of the bivalent vaccine in 12-13 year old girls has led to a reduction of around 85% of vaccine type infection in girls attending for first screen, as measured by an HPV genotyping assay with high analytical sensitivity. A significant impact of vaccine on CIN levels has also been observed. While this is hugely encouraging, the community is now faced with the challenge of how to effectively and efficiently screen vaccinated women given the radically altered prevalence of infection and disease.

Methods

Scotland has set up a national, longitudinal immunisation surveillance system to assess the impact of HPV vaccine on infection and disease, a national multi-disciplinary HPV research network (Scottish HPV Investigators Network – (www.shine.mvm.ed.ac.uk) and a national biobank of over 40 thousand samples designed to facilitate HPV research (www.shine.mvm.ed.ac.uk/archive). These endeavours have facilitated an integrated, national approach to ensure the development of primary (immunisation) and secondary (screening) disease prevention strategies are not considered in isolation. One strand of the associated research is to determine the impact of immunisation on the applicability and performance of primary cervical screening using molecular HPV testing through (1) the comparison of viral outcomes using epidemiologically orientated tests vs clinically validated tests, in immunised cohorts and (2) the assessment of performance of clinically validated HPV tests in immunised cohorts for the detection of CIN2+.

Results

In vaccine surveillance studies, viral prevalence, as defined by epidemiologically orientated assays, may underestimate the impact of that HPV immunisation on clinically relevant levels of infection (CIN2+). While sensitivity and negative predictive value of primary HPV testing for CIN2+ remains high in immunised women; positive predictive value and specificity reduces – emphasising the need for effective triage(s). The seven-fold reduction of HPV 16/18 in immunised women may limit the utility of 16/18 typing as a triage strategy of HPV positive, vaccinated women.
Conclusion

Contemporary data which reflect the level and composition of both type specific HPV infection and associated disease in immunised women is essential to support the further planning of services for appropriate cervical disease management.
Microbiome and metabolome profiles associated with HPV persistence and clearance

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Background / Objectives

To examine the role of the vaginal microbiome (VM) and its associated cytokine and metabolome profiles in a prospective study of HPV 16 acquisition, persistence and clearance.

Methods

Four visits from 13 women were selected at time of: HPV16 negativity, acquisition, persistence and prior to clearance. (---++--) Cervicovaginal lavage samples were examined for cytokine profiling using Luminex microbiome profiling using 16S rRNA analysis and metabolome profiling. Visits excluded if noted pregnancy, STI or recent antibiotics/intravaginal medication reported. Microbiome community states types (CST) were identified.

Results

No clear CST appeared associated with acquisition, persistence, or clearance of HPV16, however, significantly greater microbiome diversity was observed during persistence(figure 1). CST-III appeared immune activated with significantly higher levels of cytokines (figure 2). Metabolomic analysis found persistence associated with higher levels of mannose, important in innate immune responses, cadaverine, a genotoxic by-product released by anaerobes and nicotinamideribonucleotide(NMN), carcinogen metabolite of nicotine. G. vaginalis was associated with higher levels of mannose and NMN.

Conclusion

HPV16 persistence results in increased microbiome diversity, a hallmark of VM dysbiosis and activated innate immune states which if chronically present are associated with genotoxic products capable of inducing cancer.
The vaginal microbiota after excisional treatment for cervical intraepithelial neoplasia

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Background / Objectives

Background: The vaginal microbiota (VMB) is usually Lactobacillus spp. dominant appears to protect the female reproductive tract against infections including HPV. CST (community state type) III and the high-diversity VMB deplete of Lactobacillus spp. CST IV have both been associated with higher rates of HPV acquisition, persistence and increased severity of cervical intraepithelial neoplasia (CIN). These CST’s have also been associated with pre-term birth (PTB); a known complication of excisional treatment. Furthermore women with a history of treatment are at a higher risk of future invasive cervical disease, which could also be linked to VMB composition.

Objectives: To investigate the impact of excisional treatment for CIN on VMB composition.

Methods

Material and Methods:


Interventions: Vaginal swabs collected immediately prior to treatment, and at 6 month follow-up. Bacterial DNA was extracted and sequenced using the Illumina MiSeq platform.

Analysis: Hierarchical clustering of sequence data was used to examine bacterial species classification data, and linear discriminant analysis effect size (LEfSe) to identify biomarkers.

Results

One hundred and three women provided both pre- and post-treatment samples. Excisional treatment did not significantly alter the distribution of CSTs within the cohort, and diversity remained significantly greater compared to normal, healthy untreated controls. There was no association with post-treatment CST and HPV status. LEfSe identified Streptococcus agalactiae (Group B streptococcus) to be significantly overrepresented in post-treatment samples.
Conclusion

Excisional treatment does not appear to have a significant impact on VMB composition. CST III and IV remained at a higher prevalence than in a normal control population. These results suggest that the increased prevalence CST III and IV in women with CIN may be due to intrinsic host factors rather than as a result of disease, and these intrinsic factors may also predispose them to PTB and risk of disease recurrence. Furthermore, Streptococcus agalactiae which has been associated with PTB risk, may add to the risk in this patient cohort.

References


MICROBIOME OF HPV POSITIVE AND NEGATIVE PLACENTA

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Background / Objectives

Prenatal transmission of human papillomavirus (HPV) may occur via the placenta. HPV infection has been suggested to elicit adverse effects on pregnancy. Eleven studies have reported the prevalence of HPV DNA in the placenta to be 3-75%. The review by Ambuhl et al (2016) reported an overall HPV prevalence of 8.3% in normal full-term pregnancies.

Methods

Recently, the paradigm of sterile fetal life has been challenged. It has been suggested that the healthy human placenta may have a distinct microbiota (Aagaard et al., 2014; Collado et al., 2016). There are experimental data to suggest that virus-bacterial interaction in the placenta may lead to detrimental fetal and maternal outcomes (Mor and Kwon, 2015). Furthermore, changes in vaginal microbiota may be involved in HPV acquisition and persistence.

Conclusion

As of now, nothing is known about the association between HPV infection and the recently discovered placental microbiome. In this presentation, data regarding the influence of the HPV status on bacterial microbiota composition in the placenta will be discussed.

References

Aagard et al., The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237a65


Collado et al., Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep 2016;6:23139.
DNA methylatin markers for the detection of anal (pre)cancer in HIV+ men who have sex with men

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Background / Objectives

Anal cancer is an increasing problem in HIV+ men who have sex with men (MSM) and like cervical cancer caused by high-risk HPV and preceded by precursor lesions: anal intraepithelial neoplasia (AIN; graded 1 to 3). Currently, screening for AIN by high-resolution anoscopy (HRA) leads to over-referral and overtreatment. DNA methylation analysis is a promising pre-screening tool to detect cervical (pre)cancer, but has not been studied in the clinical management of anal (pre-)cancer in HIV+ MSM. We set out to find methylation markers that enable the detection of anal cancer or AIN in need of treatment.

Methods

A series of FFPE tissue samples of HIV+ men with anal squamous cell carcinoma (SCC; n=18), AIN3 (n=24), AIN2 (n=40) and men without evidence of AIN2 or worse (normal + AIN1; n=29) were analysed for DNA methylation of six genes known to display hypermethylation during HPV-induced carcinogenesis using quantitative methylation-specific PCR (qMSP). Univariable and multivariable logistic regression was used to determine the performance of the methylation markers for the detection of high-risk lesions.

Results

Methylation levels of all six genes increased significantly with severity of disease, with up to 95% positivity in SCC. Analysis of methylation marker combinations yielded an area under the ROC curve of 0.85 for detecting AIN3 or worse.

Conclusion

We identified a panel of methylation markers that provide an attractive triage tool to discriminate HIV+ MSM with clinically irrelevant precursor lesions (low cancer risk) from those with lesions in need of treatment.
Vaccination to prevent anal HPV infection

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Background / Objectives

Unlike cervical HPV-associated conditions, those of the anal region are increasing in all jurisdictions studied. Furthermore, treatment of anal high grade squamous intraepithelial lesions (HSIL) may be associated with considerable morbidity and high rates of recurrence.

Prevention of anal HPV infection and related conditions is currently dependent on the administration of prophylactic vaccination to individuals, ideally before the onset of sexual activity. There are currently three options: bivalent (bHPV- targeting HPV 16 and 18), quadrivalent (qHPV - targeting HPV 6,11,16 & 18) and nonavalent (nHPV - targeting HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) vaccines.

Much of the research on the efficacy, safety and immunogenicity of these vaccines has been done in the context of cervical disease. However, there are significant differences between the natural history of cervical and anal HPV infection. Much less is known about how anal HPV-related conditions behave in the context of both genders, men who have sex with men, and the immunosuppressed.

Methods

Results

There has been only one published large-scale phase randomised controlled trial of the efficacy of prophylactic HPV vaccines in preventing anal HPV-related disease. In this, the rates of grade 2 or 3 anal intraepithelial neoplasia related to infection with HPV-6, 11, 16, or 18 were reduced by 54.2% in the intention-to-treat population and by 74.9% in the per-protocol efficacy population. Persistent anal infection with HPV-6, 11, 16, or 18 infections were reduced by similar amounts.

Conclusion

This presentation will review relevant data from other, related, studies and assess what we can infer regarding the use of prophylactic HPV vaccination in the context of prevention of anal HPV-related disease in other populations.

It will conclude with a review of clinical scenarios where vaccination to prevent anal HPV infection may be considered outside existing programs.
SS 10-04
HIGH RESOLUTION ANOSCOPY AND MANAGEMENT OF ANAL INTRAEPITHELIAL NEOPASIA

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Background / Objectives

Anal squamous carcinoma is potentially a preventable disease. In the absence of primary prevention through HPV vaccination, prevention of HPV-related cancers, such as cervical cancer, are reliant upon the detection of precancer stage through screening and subsequent treatment of the precancer. High resolution anoscopy (HRA) enables the detection and management of anal high-grade squamous intraepithelial lesions (HSIL) in the field of anal cancer.

Methods

Newly published data relating to anal neoplasia field was searched and analysed.

Results

There is now international consensus in defining the standards for practice of HRA. Some recent data suggest that a proportion of untreated anal HSIL may spontaneously regress while other data suggest anal HSIL can lead to invasive anal carcinoma. A number of topical and ablative modes of treatment of anal HSIL are available. We will discuss aspects of persistence / progression vs regression in this presentation. A number of factors that may impact on progression will also be discussed. Management of multizonal anogenital neoplasia in women will additionally be presented.

Conclusion

A number of new developments have taken place in the field of anal neoplasia resulting in better understanding of the disease and its management.
NOVEL THERAPIES FOR AIN

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Background / Objectives

The incidence of anal cancer in the general population is higher among women than men, and has been rising steadily since the 1970s. Certain at-risk groups, including HIV-infected men and women are at especially high risk of anal cancer, and among HIV-infected men who have sex with men, the incidence of anal cancer may be as high as 131/100,000. Like cervical cancer, anal cancer is caused by HPV and is preceded by HPV-associated high-grade squamous intraepithelial lesions (HSIL). Like cervical cancer, anal cancer may be preventable through identification and treatment of anal HSIL.

Methods

Current methods of treatment for anal HSIL include various methods of physical removal (e.g., surgical excision, hyfrecation, infrared coagulation, laser) and use of topical creams (e.g., 5-fluorouracil, imiquimod). These are often painful and have limited efficacy. High rates of metachronous disease reflect persistence of an HPV reservoir capable of reactivation and producing new disease, and/or inability to completely clear existing lesions.

Results

Improvements in understanding the biology of HPV infection and its role in pathogenesis of anal HSIL are leading to new approaches to treating anal HSIL. These include systemic approaches such as therapeutic vaccines targeting cells expressing HPV proteins such as E6 or E7. The anatomic location of anal HSIL also offers therapeutic opportunities for topical approaches, including the possibility of repeated application. These include drugs that interfere with expression of HPV oncogenes including siRNA and clustered regularly interspaced short palindromic repeats (CRISPR) approaches. Drugs that affect cellular pathways perturbed by HPV and which are important in pathogenesis of HSIL may include those that affect methylation status and microRNAs. Drugs that may stimulate cellular immune response to HPV are also being developed such as checkpoint inhibitors that block binding of programmed death ligand (PDL)-1 binding to the programmed cell death protein (PD-1).

Conclusion

Challenges to treatment include large lesions, multifocal disease and anatomic impediments that preclude complete treatment, such as hypertrophic papillae, crypts, hemorrhoids and folds. Treatments that target HPV gene products specifically or
cellular pathways specifically altered by HPV infection, or treatments that augment cellular immune response to HPV proteins may provide an improved therapeutic index, particularly if they can be applied repeatedly with minimal inconvenience and toxicity to the patient.
EPIDEMIOLOGICAL ASSESSMENT OF HPV SAFETY – DISTINGUISHING CAUSE FROM COINCIDENCE

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Background / Objectives

Assessing the safety of vaccines is essential for maintaining public confidence and to identify true reactions which may affect the benefit-risk profile. For HPV vaccines safety has been particularly high profile in recent years with reports linking various adverse events to the vaccines. In this presentation I will cover methods for identifying safety concerns (signals), how they might be initially investigated and where necessary how/if robust epidemiological studies can be done to test hypotheses.

Methods

I will use a recent study done in England looking at HPV vaccines and Guillane Barré Syndrome as an example in which a signal was raised, data were rapidly examined and then a self-controlled case-series study done to assess the signal.

Results

In this example the ecological study showed no evidence of an increased risk, but could also not exclude a risk of up to two fold. The epidemiological self-controlled case-series study confirmed no increased risk of Guillane Barré Syndrome following HPV vaccines (incidence rate ratio in the 3 months post a vaccine dose = 1.04 (95% CI: 0.47-2.28))

Conclusion

To enable robust and timely assessment of safety it is necessary to have a variety of systems in place to detect and investigate signals. Whilst for GBS this was possible with no risk seen, other events may be more difficult to assess if there are likely reporting biases and vague case definitions.

References

POST-LICENSESURE MONITORING 9-VALENT HUMAN PAPILLOMAVIRUS VACCINE SAFETY

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Background / Objectives

The 9-valent HPV vaccine (9vHPV) was licensed in the United States in 2014 and recommended for vaccination of females and males in 2015. CDC continuously monitors vaccine safety during the post-licensure period using several systems, including the Vaccine Adverse Event Reporting System (VAERS) and the Vaccine Safety Datalink (VSD). VAERS is a national spontaneous reporting system co-managed by CDC and FDA. VSD is an active surveillance system that is a collaboration between CDC and 9 US integrated healthcare delivery systems. We describe 9vHPV safety data from VAERS, and compare with data for quadrivalent HPV vaccine (4vHPV). We also describe VSD monitoring for 9vHPV.

Methods

We searched VAERS for US reports of adverse events (AE) following 9vHPV from December 2014-December 2016. We conducted descriptive analyses of commonly reported AEs, estimated AE reporting rates, and performed clinical review of selected pre-specified conditions. In VSD, we conducted weekly near-real time sequential monitoring of 11 pre-specified health conditions (Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, anaphylaxis, stroke, venous thromboembolism, appendicitis, pancreatitis, seizures, syncope, allergic reaction, injection site reactions) among males and females aged 9-26 years using sequential analyses to detect associations.

Results

From December 2014-December 2016, approximately 19 million 9vHPV doses were distributed in the United States. VAERS received 4,348 reports following 9vHPV; 29% in females, 22% in males, 49% sex missing/unknown. Overall, 98% of reports were non-serious; dizziness (8%) and syncope (7%) were most commonly reported in males and females. Headache (42%), nausea (35%), and dizziness (28%) were the most common signs and symptoms among serious reports. In comparison for 4vHPV, between June 2006-December 2015, the majority of reports were also non-serious (93%) with similar adverse events; syncope (12%) and dizziness (12%) were the most common signs and symptoms. Headache (29%), nausea (22%), and fatigue (22%) were the most common symptoms among serious reports. To date in VSD monitoring, 671,361 9vHPV doses have been administered; syncope and local injection site reactions are the only pre-specified conditions to signal (i.e. higher than
expected numbers of adverse outcomes), both of which are known and expected AEs.

**Conclusion**

The 9vHPV safety profile to date is consistent with data from pre-licensure trials and comparable with post-licensure surveillance and epidemiologic studies for 4vHPV. Safety monitoring and evaluation of 9vHPV will continue to ensure rapid availability of information for immunization programs and the public.
SS 11-03
HPV vaccination programme in Ireland

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Background / Objectives

On the recommendations of the World Health Organization and the National Immunisation Advisory Committee the human papillomavirus (HPV) vaccine was introduced into the state school immunisation programme in 2010 to protect girls from cervical cancer. HPV4 vaccine is offered by vaccination teams to 12-13 year old girls in their first year of second level school.

Since the programme started, uptake for the completed vaccine course has been above the target of 80% with figures for 2014/2015 of 87% (the highest since the programme began). However uptake figures for 2015/ 2016 dropped to 72% and figures for the first vaccine dose in 2016/2017 are estimated at 40-50%.

This decline is related to parental concerns about HPV vaccine safety. Lobby groups of concerned parents have been established and these groups have promoted vaccine misinformation through national and local television, radio and press and have been very active on social media outlets. This is of great concern to all those involved in cancer prevention.

Methods

In early 2016, the Irish health services consulted with other interested stakeholders who agreed to take a coordinated approach to tackling negative publicity and incorrect “facts”.

Firstly, focus groups on parents’ attitudes to and experience with HPV vaccine was carried out as well as social media analysis. The results of these influenced ongoing activities including liaison with relevant bodies, revision of information materials, enhanced website materials including short videos and social media campaigns.

Regular meetings are ongoing with health representatives of all parliamentary parties and interested public representatives to ensure their ongoing support. In addition, a comprehensive training programme for a wide range of health professionals has been implemented enhanced by the production of elearning modules.
Ongoing participation continues from the wide range of very active stakeholders in television, radio and print media interviews and other forums to inform parents of the facts about the safety and effectiveness of HPV vaccine.

A catch up vaccination programme has been implemented for girls who have missed out on the vaccine.

**Results**

Public awareness of HPV vaccine has improved and media coverage is currently more balanced. Uptake figures for the 2016/17 HPV vaccination programme will be available later in 2017.

**Conclusion**

Based on previous experience, reversing the decline in vaccine uptake will require concerted long term efforts from all stakeholders to ensure girls in Ireland are protected from cervical cancer.

**References**

The rise and the fall of the Danish HPV vaccination programme, and the way ahead

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Background / Objectives

Vaccination of girls against human papillomavirus (HPV) was included in the Danish vaccination programme in 2009. Since 2013, the Danish Medicines Agency received an increasing number of reports of suspected adverse events which resulted in public concerns about vaccine safety and a dramatic decrease in vaccine uptake. By March 2017, only 35% of girls born in 2004 have received at least 1 HPV vaccine compared with a 92% uptake in birth cohorts 1998-2000. This fall represent an alarming setback for cancer prevention.

The suspected adverse events consist mainly of medically unexplained symptoms without a documented causal link to the vaccine. Massive attention in social media and news media have certainly been pivotal in amplifying vaccine skepticism in the target groups.

Methods

Actions taken in Denmark include

The establishment of treatment centres for patients who suffer from perceived adverse events

Research into the epidemiology of the crisis, including role of media and excess morbidity prior to first vaccination

Registry based research to explore any undetected side effects

A campaign to regain trust to the programme

Results

Research has, among other, suggested that females who report adverse events prior to the first HPV vaccines have increased health care seeking behavior and excess morbidity from medically unexplained symptoms compared with controls. This indicate that the symptoms are coincidental with the vaccine and not caused by it.

Conclusion

This finding provided some reassurance as regards concerns about vaccine safety, but is clearly not enough to regain the trust. A stronger communication emphasis on cancer prevention, the particular aspects of cervical cancer as a disease of young
women, and international aspects are some messages that need to be reinforced to regain trust.
Monitoring HPV vaccination in the Netherlands: data on vaccine effectiveness and safety up to 7 years post-introduction

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Background / Objectives

Implementation of routine HPV-vaccination in the National Immunisation Programme in The Netherlands is accompanied with monitoring and flanking research. HPV-vaccination using the bivalent vaccine was introduced in the National Immunisation Programme in 2010 for 12-year olds girls. A catch-up campaign was implemented for girls aged 13-16 year old in 2009. Cervical screening (from 2017 onwards on highrisk HPV) is offered to women aged 30 years and older.

Methods

Adequate monitoring of the effects of routine vaccination includes in general surveillance of vaccine uptake, safety, occurrence of the target disease, pathogen and serosurveillance. HPV vaccination differs from most other vaccine preventable diseases since the actual disease (e.g. cancer) caused by the virus takes many years. Therefore, occurrence of surrogate endpoints, including prevalent, incident and persistent HPV-infections, are currently studied in both cohorts of (un)vaccinated women and high risk groups (e.g. STI visitors) to enable measurement of early vaccine-effectiveness estimates.

Results

Results on monitoring and flanking research on HPV-vaccination will be presented up to 7 years post-vaccine implementation.

Conclusion

Given our results on high vaccine-effectiveness against early endpoints and safety of the HPV-vaccination, efforts to increase vaccine uptake need to be emphasized.
Background / Objectives

Since 2007, 75 countries have implemented human papillomavirus (HPV) vaccination programmes. It is important to examine whether the promising results from pre-licensure randomised clinical trials and predictions from mathematical models are materialising in the real world. We summarised the most recent evidence about the population-level effects of girls-only HPV vaccination programmes among girls/young women targeted for vaccination, older women, and boys/men on anogenital warts (AGW) and high-grade cervical lesions (CIN2+).
We searched Medline and Embase (2007/01/01–2016/12/16) for studies presenting changes in HPV-related outcomes between pre- and post-vaccination periods. We stratified all analysis by age/sex and performed subgroup analyses to identify the main sources of heterogeneity. We used random-effect models to derive pooled relative risk estimates.

Results

We identified 48 eligible studies from 11 high-income countries. We identified that the overall proportion of females vaccinated in the different countries (considering both vaccination coverage and number of cohorts vaccinated) was an important source of heterogeneity. Therefore, we categorised countries as having a medium/high proportion of females vaccinated (coverage ≥50% and multi-cohort vaccination) or a low proportion of females vaccinated (coverage <50% and/or single-cohort vaccination). In countries with a medium/high proportion of females vaccinated, there was a rapid and significant decline of AGW among girls/women <30 years old and boys/men <25 years old, and significant decreases in CIN2+ among girls <20 years old. Decreases in CIN2+ were also observed among women 20-24 years old after 5 years of vaccination. In countries with a low proportion of females vaccinated, significant decreases in AGW were observed after 3-4 years of vaccination among girls/women <25 years old, but there were no significant decreases among older women, and boys/men.

Conclusion

Ten years after the implementation of HPV vaccination, the promising results of randomised trials are materialising in the real world. We observed significant decreases in AGW and CIN2+ and strong herd effects, particularly in countries with a high proportion of females vaccinated. Additional surveillance data is required to examine the incremental effectiveness of gender-neutral vs girls-only vaccination programmes and the population-level impact of reduced dose schedules.
EVIDENCE FROM POST-VACCINATION STUDIES IN LOW-INCOME COUNTRIES


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Background / Objectives

Many high-income countries have established HPV vaccination programs from 2006 to 2008 and have already reported effectiveness versus individual HPV types, cervical lesions, and genital warts1. Bhutan (2010) and Rwanda (2011) were the first low-income countries to introduce national vaccination programme. These targeted 12 year-old girls (>90% coverage) and initially included catch-up campaigns (13–18 year-olds in Bhutan and ninth school grade in Rwanda). The two countries can therefore provide the earliest evaluation of such programs.

Methods

In 2013, IARC, in collaboration with the Ministries of Health, performed two school-based HPV urine surveys2. 973 female students (median age: 19 years, 5th-95th percentile: 18-22) were recruited in Bhutan and 912 (19 years, 17-20) in Rwanda. 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+ and E7-MPG.

Results

92% students in Bhutan and 43% in Rwanda reported to have been vaccinated (median vaccination age=16, 5th-95th: 14-18). HPV positivity in urine was significantly associated with sexual activity measures. 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). In Bhutan, HPV6/11/16/18 prevalence by GP5+/GP6+ was lower in vaccinated than in unvaccinated students but CIs were broad.

Conclusion

Our study supports the feasibility of urine surveys to monitor HPV vaccination and quantifies the effectiveness of the quadrivalent vaccine in women vaccinated after pre-adolescence. In the future, as vaccinated cohorts of women will predominantly represent those vaccinated in pre-adolescence in Bhutan and Rwanda, similar repeat
urine assays can be expected to detect greater increases in HPV vaccine effectiveness than in our present study. Medium/long-term monitoring in low-income countries would benefit from the introduction of HPV-based screening and the strengthening of cancer registries and mortality data. For the moment, if resources for surveillance of viral or clinical endpoint are lacking, priorities in LMICs should be monitoring coverage and side effects.

References


INFORMING STRATEGY ABOUT TARGET GROUPS: GIRLS-ONLY, GENDER-NEUTRAL, CATCH-UP AND SELECTIVE ADULT VACCINATION

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Background / Objectives

HPV vaccination programmes have been implemented in many countries, but gender- and age-specificities vary strongly. Preadolescent girls are always included into HPV vaccination programmes, but the lower and upper ages of admissibility are variable, as were the number of birth cohorts considered for catch-up vaccination at the time of vaccine introduction. Countries are gradually extending vaccine eligibility to preadolescent boys, sometimes without underlying cost-effectiveness evaluations. Selectively expanding HPV vaccination to at-risk adults (e.g. men-who-have-sex-with-men (MSM) or non/partially vaccinated women attending cervical screening) is advocated as an alternative to improve HPV prevention efforts.

Methods

We consider general principles for informing strategy about gender- and age-specificity of HPV vaccination programmes. Many of these principles are derived from analyses based on mathematical models designed to inform decision-making. Some principles relate to structural issues whereas others are regulated by model parameters.

Results

Vaccinating girls prior to sexual debut is unequivocally considered cost-effective, but findings regarding catch-up campaigns strongly depend on local cost and sexual network assumptions. Analyses of boys’ vaccination are more elaborate because these are often considered conditional on girls’ vaccination. Gender-neutral vaccination evaluations may hinge on concealed entities such as herd immunity from girls’ vaccination, etiologic fractions of non-cervical cancers considered, attribution of residual disease burden to MSM, and realistic costs of vaccine procurement and delivery. Consequently, the estimates of vaccinating boys in addition to girls are highly variable between settings and assessments. The efficacy of selective adult vaccination has been demonstrated in clinical trials, but relatively few estimates exist of the absolute health gains from vaccinating MSM or screen-eligible women. Their population impact likely depends on factors (e.g. latency, reactivation, HIV coinfection) that still may require elucidation in mathematical models.

Conclusion

The consistent estimates on cost-effectiveness of girls’ vaccination contrast with those for boys, which is partly due to the conditioning of gender-neutral on girls-only
vaccination. The health economics of selective adult vaccination are worth exploring in model-based assessments.
Prophylactic vaccination following treatment of GW and CIN

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Background / Objectives

The currently available HPV vaccines are designed as prophylactic vaccines with a type specific vaccine efficacy of almost 100%. No therapeutic effect was found in the phase three trials and no influence on the clearance of HPV infections has been reported yet. However after treatment of HPV related disease a reduction of subsequent HPV related disease was observed.

Methods

A review of the literature will be performed.

Results

A post hoc analysis of the phase 3 trials with the quadrivalent vaccine revealed a 65% reduction of recurrent high grade cervical disease of the cervix in vaccinated women after conization compared to placebo recipients. In the same trial women with HPV related vulvar disease had a reduction of subsequent HPV related vulvar disease of 35%. A very similar post-hoc analyses with the bivalent HPV vaccine demonstrated a 88% reduction of subsequent CIN2+ and a 43% reduction of CIN1+ following conization in vaccinated women compared with placebo. In the Costa Rica trial with the bivalent vaccine no effect after conization was observed. A retrospective Korean study in >700 women vaccinated after conization with the quadrivalent HPV vaccine found a reduction of two thirds of recurrent disease (7.2% vs 2.5%).

Conclusion

Available data suggest that HPV vaccines have a value as a tool of secondary prevention after treatment of HPV related disease. No direct therapeutic effect has been observed. A prospective study is ongoing and will hopefully strengthen the evidence, real world data from the Nordic countries are awaited.

References


Background / Objectives

Vaccines to prevent HPV infection were originally developed with the purpose of addressing disease of the genito-urinary tract. An additional benefit of vaccinating against HPV sub-types 6 and 11 is potential to drastically reduce the incidence of new case of recurrent respiratory papillomas.

Methods

Studies demonstrating the potential for preventing transmission of HPV in the aero-digestive tract will be presented along with the initial findings from the CDC-funded retrospective and prospective studies in the US and from Canada demonstrating early signs of reduction in incident and prevalent cases.

Results

Preliminary results from the US and Canadian RRP registry studies will be presented along with correlated data regarding reduction in ano-genital warts.

Conclusion

Wide-spread HPV vaccination covering sub-types 6 and 11 has the potential to drastically reduce the incidence and future prevalence of RRP in populations that can achieve herd immunity.
SS 13-05
Vaccination for populations at higher risk of cancer

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Background / Objectives

Prophylactic HPV vaccinations are now being offered to young girls in many countries, and other countries are on the verge of starting vaccination in girls an/or boys. Despite catch up vaccination of adolescent en young adult women at the start of the vaccination campaign, a large cohort of older women remains unvaccinated and at risk of HPV related disease/cancer. This presentation will highlight women and men at increased risk of HPV related disease later in life, who may benefit from prophylactic HPV vaccination.

Methods

In the literature many studies have shown increased risk of HPV related disease in women and men who are imunosuppressed. The literature was searched in order to find articles reporting on immunosuppression and HPV related disease, as well as articles reporting on vaccination of groups at supposed increased risk of HPV related disease.

Results

Several different groups could be identified in the literature that have an increased risk of HPV related disease. Two large cohort studies showed that women who have ever been treated for a high grade cervical intraepithelial neoplasia grade 2-3 (CIN 2-3) are at increased risk of developing HPV related disease at any site (cervix, vagina, vulva, anus and oropharyngeal). This risk is still increased, 20-25 years after having been treated. Additionally, several studies have shown that prophylactic HPV vaccination around the period of treatment of HPV related disease may prevent recurrent disease in 55-65% of cases, although one other study could not confirm these results. In men, one study (not yet published) showed benefit of HPV vaccination in men having sex with men, who were treated for anal dysplasia.

Other studies have shown that women who are immunosuppressed due to Human Immunodeficiency Virus (HIV) infections, or due to immunosuppressive drugs (Organ transplant patients, Systemic lupus erythematosus patients, other autoimmune disease patients), are at increased risk of HPV related disease. However, no studies on vaccine efficacy in these groups have been reported yet.

Conclusion
Several groups of women may be identified that have an increased risk of HPV related disease. These men/women did not receive any prophylactic HPV vaccination yet, and studies are emerging indicating vaccine efficacy in these groups. In order to further decrease HPV related diseases, these groups must be studied urgently, in order to be able to offer them prophylactic HPV vaccination on a risk based strategy.

References

Ebisch R. et al. Long-lasting increased risk of HPV-related carcinomas and premalignancies after cervical intraepithelial neoplasia grade 3: a population-based cohort study. JCO 2017 (accepted for publication)


Joura E. et al. Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data. BMJ 2012 March 27;344e


Background / Objectives

Sexual abuse of children is estimated to have a prevalence of 8-31% worldwide with slightly higher rates reported in girls than in boys. It is also thought that these estimates are much higher since the majority of abuse never is reported. Over two thirds are relatives or close family members. Other common perpetrators are often authority figures such as teachers or religious leaders. Certainly, any sexual contact can result in HPV exposure and infection including fondling. Forceful attempts at vaginal or anal intercourse can result in tears and exposure of the basal epithelium where HPV infections occur.

Methods

Literature was reviewed.

Results

The risk of acquisition may be heightened since the prepubertal and peripubertal vaginal and cervical epithelium is thin and unprotected with easy access to the basal epithelium. There is data to suggest that sexually abused persons are at high risk of anogenital cancers. Risk of cancer by age of sexual exposure is not known. However, it is plausible that the younger that a child is infected, the longer they may experience HPV persistence, a strong risk for anogenital cancers. In addition to potential HPV exposure, high risk behavior may also play a role.

Persons who have experienced childhood sexual abuse often display high risk sexual behaviors including young age at voluntary sexual activity, a high number of sexual partners, and frequent unprotected sex. Persons with history of abuse are at risk for homelessness and substance abuse leading to exchanging sex for food, shelter or money. Alcohol and drug use are also increased in this population which is associated with poor judgement when having sex while intoxicated.

Conclusion

The US Center for Disease control suggest that children with a history of sexual abuse should be vaccinated as early as 9 years of age before the likelihood of engaging in high risk behaviors. Some have suggested that in addition to sexually transmitted infection screening and prophylactic treatment, the HPV vaccine should be offered. There is no data to guide at which age this is appropriate. Expert opinion also suggest that this population should be screened for cervical cancer earlier than 21 years of age---such as 18 years. However, the risk vs benefit of this is not known specifically that the pelvic examination itself as well as the “worry” of cancer may elicit significant psychologic distress.
OVERVIEW OF THE STRUCTURE AND AIMS OF CISNET-CERVICAL

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Background / Objectives

The increasing number of cervical cancer models that have been developed and published signals a "demand" for model-based policy analyses, comparative effectiveness analyses, and cost-effectiveness analyses. Although several review papers compare model assumptions and results post-hoc, there are limited examples of rigorous comparative modeling to try and understand how differences across models comply with the evidence and impact results.

Methods

Motivated by a strong desire to address gaps in cervical cancer control and the need for formal comparative modeling, we have assembled five independent teams of cervical cancer modelers: Harvard University (USA), University of Minnesota (USA), Erasmus Medical Center (The Netherlands), and Cancer Council New South Wales (Australia); the University of Washington (USA) focuses on key questions for HIV-positive women. Our objective is to use rigorous, comparative modeling to inform effective, efficient, and equitable policies for cervical cancer control in the United States with the following specific aims: (1) to evaluate the health benefits, harms, and costs of cervical cancer prevention strategies as currently practiced, including HPV vaccination and screening; (2) to identify the most efficient and cost-effective cervical cancer control strategies, taking into consideration new and forthcoming technologies; (3) to identify the most efficient and cost-effective cervical cancer control strategies in HIV-positive women; and (4) to leverage unique opportunities afforded by the CISNET comparative modeling approach to improve translation and validation of model-based analyses of HPV and cervical cancer control. We expect to have an impact on the analytic methods of comparative modeling; the equitable distribution and rational use of new technology; the effectiveness of interventions and strategies for cancer prevention through clinical guidelines and national policies; HPV-related cancer outcomes, including reduced incidence, enhanced quality of life, improved survival, and reduced disparities; and the financial and economic profile of delivering cancer-related health services.

Conclusion
For policy makers and stakeholders to be able to use model-based results in decision making, there needs to be transparency, consensus on analytic standards, some degree of harmonization across studies, and explanations of how and why results may differ. CISNET provides an unprecedented opportunity to not only fill this void in cervical cancer modeling but also address key scientific questions that move our field forward.
Comparative modelling - how do the outputs from current state-of-the-art models compare

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Background / Objectives

How cervical screening interferes with cervical cancer and cervical cancer death is a complex matter. Therefore, also the models we develop to help optimizing screening interventions, are not simple. Nor is it simple to compare the models. Especially where the models differ in structure, parameters act differently and cannot be directly compared. This most often is the case for the parts in de models where unobservable processes are described: the natural history of disease and how screening and subsequent treatment interfere with that history. Any tools to clarify similarities and differences between models are welcome.

Methods

Cancer incident and death cases after screening participation have 3 possible origins: 1) the cancer-precursor was not present yet at the time of the screening, 2) the precursor was missed by the screening, or 3) treatment of the screen-detected precursor was not successful in preventing further cancer and cancer death. In models, we can discern these cases and in CISNET, we developed the MCLIR method to do so. It is based on comparing model output of specially designed - partly hypothetical - screening scenarios. We considered 3 of the CISNET cervical models, as they were developed independently before CISNET started, for the USA, Australia and the Netherlands. We compared them for cytology screening effectiveness after a one time screening, and compared the contribution of the three reasons for screening failure.

Conclusion

Overall, the patterns in incidence and mortality reduction after screening were quite similar between the models. This was particularly true for the role of the duration of cancer precursors. The models differed somewhat in the ability of cytology to detect these precursors. The models differed most in how successful treatment of screen-detected conditions was in preventing further morbidity and mortality.
LEVERAGING SIMULATION MODELS TO EXPLORE THE NATURAL HISTORY OF CERVICAL CARCINOGENESIS: A CISNET COMPARATIVE MODELING ANALYSIS

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Background / Objectives

The natural history of human papillomavirus (HPV)-induced cervical cancer is largely unobservable, yet the length of time spent with preclinical disease impacts the effectiveness of alternative screening policies. Mathematical simulation models are increasingly being used to capture the complex natural history processes to project the health benefits and economic consequences of alternative cervical cancer prevention approaches.

The cervical cancer working group of the Cancer Intervention and Surveillance Modeling Network (CISNET) represents four independently developed microsimulation models of the natural history of cervical carcinogenesis. We aimed to perform a comparative modeling exercise to explore the differences in natural history of cervical cancer by characterizing the age of acquisition of the causal HPV infection and identifying the implied dwell times for distinct preclinical phases of cervical disease among women that developed cervical cancer.

Methods

We used the four CISNET-cervix microsimulation models (Cancer Council New South Wales, Erasmus Medical Center, Harvard, and University of Minnesota) to project outcomes for a hypothetical cohort of individuals. The natural history models were calibrated to match observed data on age-specific HPV prevalence and HPV type distribution in precancer and cancer, but varied in their underlying structure and assumptions of the carcinogenic process. For women with a cervical cancer diagnosis and in the absence of screening or vaccination, we calculated the age of acquisition of the causal HPV infection, and dwell times associated with three phases of cancer development: 1) “HPV dwell time”, defined as the time from the acquisition of an HPV infection to development of a high-grade precancer, 2) “precancer dwell time”, defined as the time from the development of a high-grade precancer to asymptomatic cancer development, and 3) “sojourn time”, defined as the time from asymptomatic cancer development to clinical detection. Conditioned on developing cancer, we enumerated these estimates for: 1) all high-risk HPV infections, 2) HPV-16, 3) HPV-18, and 4) other non-HPV-16/18 genotypes.
Conclusion

Our findings have important implications for prevention policies, for example catch-up vaccination, vaccination at older ages (HPV-FASTER) and screening interval. The comparative analyses address concerns about model transparency and highlight important structural differences. As the complexity of microsimulation models increases, understanding the impact of differences between model structures can elucidate important drivers of cervical cancer policy and provide guidance for areas of future research.
Background / Objectives

Benign hysterectomy rates have an important effect on the population who are truly at risk of cervical cancer. Cervical cancer incidence rates that take into account hysterectomy prevalence have been shown to be much higher than those which are not hysterectomy-adjusted, especially for older women. These findings can affect conclusions about optimal cervical screening policies, especially decisions about when older women can exit screening. Policy evaluation models require estimates of hysterectomy incidence (not prevalence) in the population, but some routine data under-report hysterectomy incidence by excluding outpatient procedures. Additionally, in the USA hysterectomy procedures are still considered to be over-utilized and alternatives to hysterectomy underutilized; as alternative procedures become more widely used, hysterectomy rates may continue to decline, which will impact future rates of cancer incidence.

Methods

We performed a systematic search of Medline, Embase, Premedline, and Cochrane Central databases for articles on incidence, prevalence and trends in hysterectomy in United States. Data sources and references in retrieved articles were searched for further relevant data. Data were extracted by age, year, hysterectomy type (total or subtotal), and procedure setting (inpatient or outpatient). State- or insurance-based data were used to estimate the fraction of hysterectomies performed as an outpatient procedure over time.

Using these identified data sources, we re-estimated hysterectomy prevalence and incidence in the USA, taking into account hysterectomies performed as an outpatient procedure that are omitted from national datasets such as the National Inpatient Sample and National Hospital Discharge Survey. Resulting hysterectomy prevalence estimates were validated by comparing with self-reported survey data from the Behavioral Risk Factor Surveillance System. We then projected hysterectomy incidence rates into the future assuming 1) rates are unchanged from current observed rates and 2) rates continue to decline as cervix-preserving alternatives to hysterectomies become more commonplace. These re-estimates of hysterectomy prevalence were also used to re-estimate cervical cancer incidence by age and over time, including projections of cervical cancer rates out to 2050.
Conclusion

These estimates and future projections of hysterectomy incidence will be important in informing optimal cervical cancer prevention policies. Declining hysterectomy rates have implications for cervical cancer prevention over the coming decades as hysterectomy procedures become replaced with less invasive alternatives.
EXPLORING THE ASSOCIATION BETWEEN HPV AND HIV IN KWAZULU-NATAL, SOUTH AFRICA: A MICROSIMULATION STUDY

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Background / Objectives

KwaZulu-Natal is heavily affected by HPV and HIV. As these viruses are potentially important drivers in each other’s epidemic, interventions targeting one of them might have spillover benefits to the other. Mathematical simulation models can be used to explore the dynamics of HPV and HIV in this high endemic area. These explorations were performed as part of a comparative modeling exercise of three mathematical models of the cervical cancer working group of the Cancer Intervention and Surveillance Modeling Network (CISNET).

Methods

One of the models included in the comparative modeling exercise is the microsimulation model STDSIM. We used a previous application of STDSIM for HIV in KwaZulu-Natal, and incorporated the transmission of HPV. We calibrated natural history parameters of HPV, and co-factor effects between HIV and HPV to reproduce observed relationships between infections. We determined the impact of HPV vaccination (90% efficacy, 60% coverage) and changing eligibility for HIV treatment on HPV and HIV prevalence over a period of 20 years since the implementation of HPV vaccination in our model.

Results

We were able to reproduce the observed HIV prevalence, the proportions of HIV positive and negative women with HPV, and the association between HPV and HIV. Preliminary results from the STDSIM model show that offering HPV vaccination reduces HPV prevalence from 26.8% to 26.0% and HIV prevalence from 22.1% to 20.8% after 20 years. However, spillover effects of HPV vaccination in our model diminished under guidelines of ART for all HIV-infected people. Finally, our analyses show that HIV interventions substantially impact HPV burden, as ART for all HIV-infected people is expected to decrease HPV prevalence from 26.8% to 23.5% after 20 years.

Conclusion

Our model suggests that interventions targeting HIV also impact HPV and thus the cervical cancer epidemic. In contrast, the impact of HPV vaccination on HIV incidence is limited. Future research should explore different intervention
combinations to optimize HPV and HIV prevention in high endemic settings, and identify structural differences between the mathematical models.
Background / Objectives

Cervical cancer screening involves a multi-step process that includes initial screening, follow-up testing, diagnosis, and treatment of precancer. "Leakages" in this process can diminish the effectiveness and cost-effectiveness of screening. Model-based evaluations of the long-term health impact and cost-effectiveness of alternative cervical cancer prevention strategies in the United States often make optimistic assumptions of perfect coverage and follow-up compliance. However, there is evidence that cervical cancer screening practice is imperfect and inefficient. Integration of this evidence into policy models are imperative to understand the real-world impact and cost-effectiveness of screening strategies.

Methods

We describe the challenges of estimating the practice of cervical cancer screening in the United States. We estimate the frequency with which women in the U.S. receive cervical cancer screening, as well as compliance to referrals for diagnostic procedures and precancer treatment using different sources of empiric data, including a national survey in which respondents self-report on screening practices, large health organizations, and a state-wide lab-based screening registry, the New Mexico HPV Pap Registry (NMHPVPR). Using a microsimulation model, we provide examples of how different inputs for screening practice yield different estimates of the long-term health impact and cost-effectiveness.

Results

We found that several sources of national surveys reported on the frequency of cervical cancer screening cross-sectionally, but longitudinal data and data on follow-up visits were limited. Screening intensity reported in the national surveys were much higher than suggested by lab-based data in the NMHPVPR over the same time period. National surveys typically did not collect information beyond the initial screening visit. When we integrated data from the NMHPVPR on the full spectrum of cervical cancer screening into the microsimulation model, including both underutilization and overutilization of diagnostic procedures and precancer treatment, we found that estimates of life-years gained and costs deviate widely from scenarios under ideal assumptions of perfect coverage and compliance.

Conclusion

The challenges in modeling cervical cancer screening practice limit our ability to estimate the true health and economic impacts of screening. Understanding patterns
of screening in different health care settings is a priority for the CISNET-Cervical working group to overcome these challenges and identify ways in which to make improvements to maximize the impact of cervical cancer screening in the United States.
Background / Objectives

Cervical cancer incidence has decreased by more than 50% in Sweden since late 1960s, when a cervical screening program was introduced. However, during the last decade the incidence plateaued and from 2014 a clear increase in incidence was observed. Using recently available data (June 2017) the objective of this study was to investigate time trends in cervical cancer incidence in Sweden during 2005-2015 by age, histology and geographical locality.

Methods

Age standardized incidence was calculated yearly and for three time periods (2006-2009, 2010-2013, 2014-2015) and the mean annual change in cervical cancer incidence during 2005-2015 was estimated in linear regression models.

Results

The incidence was stable from 2006-2009 to 2010-2013 at around 9.5 per 100,000 women, while in 2014-2015 an increase by around 20% was seen to 11.5 per 100,000 women. The mean annual change in incidence during 2005-2015 was 1.7%. At ages below age 30 the mean annual change was 6.4% and at ages 30-44 the mean annual change was 3.8%. For older age classes (45-59, 60-74, 75+) no statistically significant change was observed. For squamous cell cervical cancer the pattern was the same with an ever stronger mean annual increase at ages below age 30 (8.6%), although in absolute terms this represents a fairly small change due to a low incidence at ages below 30. For adenocarcinoma there was a mean annual increase of 4% at ages 30-44, but not statistically significant change for any other age class. Overall increase in cervical cancer incidence was strongest in seven middle sized Swedish counties (mean annual changes 7-8%), while for the three largest cities (Stockholm, Gothenburg and Malmö) the incidence was stable over time.

Conclusion

The reason for the increase in incidence during this time period is not yet known, but may be related to the regional organization of the cervical screening program in Sweden and/or an increase in the underlying risk of cervical cancer.
SS 17-02
HPV 16 variants distribution in ano genital cancers

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Background / Objectives

Human papillomavirus (HPV)16 is the most oncogenic HPV, responsible for most papillomavirus-induced anogenital cancers. HPV16 causes 70% of invasive cervical cancers (ICC) worldwide and a larger proportion of HPV related tumors of the anogenital region. HPV16 genetic diversity together with host genetics and target tissue largely determine the chances to trigger carcinogenesis.

We have explored by sequencing and phylogenetic analysis the viral variant lineages present in 692 HPV16-monoinfected invasive anogenital cancers (cervix, vulva, vagina, ano, pene) from Europe, Asia, and Central/South America.

Methods

Tumor samples analysed in this study stem from a Formalin Fixed Paraffin Embedded repository from the Catalan Institute of Oncology (ICO), Barcelona, Spain. All samples were tested for the presence of tumour tissue, for the presence of HPV DNA using the SPF10-LiPA25 protocol (version 1; Laboratory Biomedical Products, Rijswijk, Netherlands) and (posar algo de com s'ha fet la determinació de les variants). We have assessed the contribution of geography, anatomy and histology (only ICC) to the differential prevalence of HPV16 variants Further, phylogenetic relationships of the E6, L2 and URR sequences generated from the samples in the global context of HPV16 genetic variability were inferred using an Evolutionary Placement Algorithm on RAxML_v7.2.8 with the GTR+Γ model.

Results

The most prevalent variant (above 70% prevalence) in all regions and in all locations was HPV16_A1-3, except in Asia, where HPV16_A4 predominated in anal cancers. HPV16_A1-3 variants were more prevalent in SCC while HPV16_D variants were increased in glandular invasive cervical cancer. We confirm further a non-random geographical structure of the viral variants distribution and histology in ICC.

Conclusion

There was a wide variation of HPV16 variants prevalence in anogenital cancers, partly explained by the geographical origin of the sample and only marginally explained by the anatomical location of the lesion, suggesting that tissue
specialization is not essential evolutionary forces shaping HPV16 diversity in anogenital cancers.

References


LIMITED HPV16 GENETIC VARIABILITY WITHIN WOMEN WITH MULTI-SITE INFECTIONS, AND LARGE VARIABILITY BETWEEN WOMEN

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Background / Objectives

We examined HPV16 whole-genome sequences of 56 paired samples from 54 women with multiple-site HPV16 infections to evaluate viral isolates within a woman at multiple anatomical sites. We additionally evaluated cervical viral isolate diversity among 59 women in the Costa Rican vaccine trial and 3,215 women in the National Cancer Institute's Kaiser Permanente NCI-KPNC Persistence and Progression (PaP) Cohort.

Methods

We whole-genome sequenced HPV16 DNA using next-generation sequencing. We analyzed 56 cervical-anal (N=42), -vulvar (N=12) or -oral (N=2) pairs. These data allowed us to determine the number of unique HPV16 genome sequences, or "isolates," in each population.

Results

For paired CVT samples within a woman, the viral isolates were the same in 85.7% of pairs (71.4% were exactly the same HPV16 genome and 14.3% differed by 1-2 nucleotides). 12.7% of pairs had different viral isolates (differing by >2 nucleotides). Between the women's cervical samples in CVT, we observed the opposite pattern, 79.7% of viral isolates were unique and 20.3% of viral isolates were the same (shared). This high cervical isolate diversity between women was confirmed in 3,215 women from the PaP cohort: 76.0% of HPV16 sequences were unique (2,445 isolates), 24% were shared among women.

Conclusion

There was no evidence of tissue tropism within the paired samples and limited isolate diversity within a woman. In contrast, few viral isolates are shared between women,
indicating a huge number of circulating viral isolates. This level of detail can improve the epidemiologic study of viral acquisition and persistence/clearance/re-appearance.
New insights into the natural history of anal HPV infection: long term data from the SPANC study

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Background / Objectives

Anal cancer shares many similarities with cervical cancer and it has therefore been proposed that cervical HPV management paradigms might successfully be applied to people at high risk of anal squamous cell cancer (ASCC). However, early evidence suggests that approaches developed for the cervix may need to be modified for the anus.

The Study for the Prevention of ANal Cancer (SPANC) set out to gain an understanding of the natural history of anal HPV infection and associated conditions. This could then provide an evidence basis for the development of interventions designed to reduce the clinical burden of ASCC.

Methods

From 2010, a total of 617 gay and bisexual men were enrolled into a study requiring five visits over three years. At each visit, participants had an anal swab tested for cytological changes and HPV detection using the Linear Array method. High Resolution Anoscopy was performed at each visit and biopsies taken from any areas suspicious of High grade Squamous Intraepithelial Lesions (HSIL). Unlike most other studies, participants were not routinely treated for any detected clinical abnormalities.

Results

86.7% of participants had ≥1 anal HPV type detected and 29.4% had HPV16. Over one third of participants (35.3%) had no nonovalent prophylactic HPV vaccine (9vHPV)-preventable HPV types detected. HPV16 was the most common prevalent HPV type (29.4%). The incidence of anal HPV16 infection was 3.64 per 100 per year. The incidence of infection with one or more 9vHPV targeted genotypes was 17.78 per 100 per year. There was no difference in incidence of HPV16 or other 9vHPV type by age or HIV status. At baseline, 36.7% had cytological and/or histological HSIL. The number of lifetime receptive anal partners, HIV status and HPV16 were all associated with the detection of HSIL-AIN3.

HPV16 was more likely to persist at six months, compared to other high risk HPV (HRHPV) genotypes. In men with HSIL at baseline, 22% cleared their HSIL at one year. Clearance of HSIL was associated with younger age, but not HIV status. Persistence of HSIL was strongly associated with the detection of any high risk HPV and HPV16 at baseline and persistent infection with these genotypes. Persistence of HSIL was more likely to occur in those with larger lesions.
Conclusion

The high percentage of individuals with prevalent anal high risk HPV and HSIL, together with the transient nature in some participants, suggests that further risk stratification techniques might be helpful in identifying those at highest risk of developing ASCC. Monitoring and treatment resources could then be more specifically targeted at such individuals, reducing unnecessary investigation and treatment.
THE DISTRIBUTION OF HUMAN PAPILLOMAVIRUS GENOTYPES AMONG CERVICAL CANCER CASES IN EUROPE

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Background / Objectives

Cervical cancer, which is principally caused by high-risk (HR) HPV types, is the second-most frequent cancer among women in Europe, associated with more than 24,000 deaths annually. This study aims to collate published information on HPV genotype distribution among invasive cervical cancer (ICC) cases in Europe.

Methods

Systematic literature searches were conducted in PubMed/Medline and EMBASE databases to identify complete publications reporting type-specific prevalence of HPV infections in histologically confirmed ICCs among European women. Original studies, published from January 2000 and available in October 2016 on PubMed/MEDLINE or EMBASE, that reported on type-specific prevalence of types 16 and 18 and at least one other of types 31, 33, 45, 52, 58 were included. Key exclusion criteria were special populations (e.g., immunocompromised, HPV-positive only), not English, population < 50. Study design, country, population characteristics, sample type, HPV assay and HPV data were extracted.

Results

Fifty eligible publications were identified reporting on type-specific HPV prevalence in 11,876 histologically confirmed ICC cases. The United Kingdom and Italy contributed the largest number of studies (7 each), reporting on 2,175 and 1,021 cases, respectively. HPV DNA was detected in 10,685 (90.0%) of ICC cases. HPV 16 was the most common type, with a prevalence of 59.2% (7,026/11,876) across Europe, ranging from 56.6% in Northern to 67.1% in Eastern Europe. HPV 18 was the second most prevalent type (12.6%, 1,477/11,744), ranging from 8.4% in Eastern Europe to 16.4% in Northern Europe. The next most frequently detected HPV types were HPV 33 (5.2%, 566/10,823), HPV 45 (4.4%, 507/11,568), HPV 31 (4.0%, 443/11,000) and HPV 52 (2.1%, 212/10,271), followed by HPV 39 (1.5%, 94/6,137), HPV 35 (1.5%, 94/6,380), HPV 58 (1.4%, 132/9,651), HPV 51 (1.3%, 118/8,969) and HPV 56 (1.2%, 101/8,214).

Conclusion

HPV genotypes 16 and 18 dominated in ICCs from European women, followed by HPV 33, HPV 45 and HPV 31. In subsequent analyses, potential sources of
heterogeneity between studies, including geographical region, histological classification, sample collection method, and year of publication, will be evaluated
ESTIMATING THE POPULATION ATTRIBUTABLE FRACTION OF ALL HPV DNA DETECTIONS DUE TO PARTNER DEPOSITION: HITCH COHORT STUDY

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Background / Objectives

Detection of HPV DNA in genital samples may not always reflect true infections but may reflect depositions from recent sex with infected sexual partners. We estimated the fraction of all HPV DNA detections potentially attributable to deposition using excess type-specific HPV partner concordance associated with recent vaginal sex in a population of young heterosexual couples.

Methods

Women aged 18-24 and their male partners aged ≥18 were recruited into the HITCH study during 2005-2010 in Montreal, Canada. We used PCR to test both partners’ baseline genital samples for 36 HPV types. We analyzed the cross-sectional association between number of days since last vaginal sex and type-specific HPV DNA concordance (probability both partners are HPV positive for the same type if one partner is positive) using log-linear multivariate models and GEE to account for correlations between HPV types. We calculated the population attributable fraction of HPV DNA detections associated with recent vaginal sex.

Results

Type-specific HPV concordance was 42.4% in partnerships where at least one partner was HPV DNA positive for that type. Type-specific concordance was 26.5% higher (95%CI 12.9-40.0%) between partners who last had vaginal sex 0-1 than partners who last had vaginal sex 8-14 days ago, and was 22.6% higher (95%CI 9.7-35.6%) between partners who never use condoms than between partners who always use condoms, even after adjustment for frequency of vaginal sex and cumulative number of sex acts since the start of the partnership. Partners who never used condoms had 42.1% higher type-specific concordance the day after vaginal sex than ≥4 days later. Under the assumption that the adjusted excess concordance between partners who had vaginal sex in the past week reflects deposition, we
estimated that 14.1% (95%CI 6.3-21.9%) of all type-specific HPV DNA detections in our cohort were depositions due to vaginal sex in the past week.

Conclusion

A substantial proportion of HPV DNA detections may be depositions due to recent vaginal sex and lack of condom use in sexually active young adults. Condom use prevents deposition and should be recommended before HPV DNA testing and for the prevention of HPV transmission.
SS 17-07

BIVALENT VACCINATION LEADS TO REDUCED VACCINE TYPE VIRAL LOAD IN INCIDENT INFECTIONS

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Background / Objectives

Bivalent HPV vaccination has shown strong protection against persisting HPV16 and 18 infections, but less against incident infections. This study aimed to assess the effects of bivalent vaccine on the viral load (VL) of HPV16 and 18 in an observational cohort study. In addition, VL assays were developed for HPV6, 11, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 to assess possible vaccine cross-protective effects.

Methods

Samples were obtained annually from an observational cohort study with vaccinated (3V) and non-vaccinated girls (0V). Up to eight samples were available per study participant. Genotyping occurred via the SPF10-DEIA-LiPA25 platform. Type-specific (TS) qPCR assays were developed for HPV6, 11, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66. Sensitivities of TS assays were in line with SPF10 or better. Participants were included in analysis if TS HPV DNA negative at baseline. Group differences in TS VL of 3V and 0V participants were assessed by Mann-Whitney testing. Persistent infection VL measurements were pooled per year of duration for each type and median was plotted with 95% CI. HPV TS incidence and persistence rates (IR, PR) were calculated by generalized estimating equation to assess protective vaccine effects.

Results

In total 555 women (50.1% 3V; 49.9% 0V) with TS incident and 451 (49.9% 3V; 50.1% 0V) with TS persistent HPV infections were included in VL analysis. For incident infections, vaccine type VL values were lower in the 3V group (HPV16 p=0.04, HPV18 p<0.01). (Borderline) lower VLs were found in 3V for HPV6 (p=0.08), 51 (p=0.07) and 59 (p=0.01). No significant effects on VL were found for other HPV types. Persistent infections showed similar kinetics over time for both groups for any HPV type.

Reduced VL was associated with reduced IR of HPV16 and reduced PR for HPV16 and HPV18 among 3V, implying protection. Despite effects on VL for HPV6 and 51, no changes in IR or PR were observed for 3V, suggesting no cross-protective vaccine effect. Interestingly, for HPV59 a reduced VL seemed associated with an increased PR among 3V. For HPV31, 33, 35 and 45 possible cross-protective effects
were observed, as reduced IR were found in 3V for these types, as well as a reduced PR for HPV31. However, no link with VL was identified.

Conclusion

Reduced VL for both HPV16 and HPV18 was found in vaccine recipients, possibly explaining lower occurrences of persistent HPV16 and 18 infections among 3V. Cross-protective effects of the vaccine were not associated with VL measurements, possibly due to the limited size of the dataset. Kinetics of VL in persistent infections did not differ between 3V and 0V for any HPV type, but number of infections was limited per HPV type.
SS 17-08
VAGINAL AND VULVAR INTRA-EPITHELIAL NEOPLASIA IN YOUNG WOMEN

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Background / Objectives

To estimate the proportion of vulvovaginal lesions in young women attributable to human papillomavirus (HPV) types preventable by the 9vHPV vaccine.

Methods

Prospectively diagnosed vulvar and vaginal low- and high-grade squamous intraepithelial lesions (LSILs and HSILs, respectively) among 8,798 women 15–26 years old enrolled in the placebo arms of 2 phase 3 randomized HPV-vaccine trials were analyzed for the presence of 14 HPV genotypes (6/11/16/18/31/33/35/39/45/51/52/56/58/59).

Results

Overall, 40 vulvar LSILs, 46 vulvar HSILs, 118 vaginal LSILs, and 33 vaginal HSILs were detected ~4 years of follow-up. At least one of the 14 types was detected in 72.5%, 91.3%, 61.9%, and 72.7% of these lesions, and multiple HPV types were detected in 40.3%, 30.4%, 24.1%, and 45.2% of the HPV-positive lesions, respectively. After accounting for co-infections, 60.0-67.5% of vulvar LSILs, 76.1-91.3% of vulvar HSILs, 27.1-43.2% of vaginal LSILs, and 42.4-60.6% of vaginal HSILs were attributable to 9vHPV vaccine types. Among the HPV-positive lesions, 89.4% of vulvar LSILs, 100% of vulvar HSILs, 56.0% of vaginal LSILs, and 78.3% of vaginal HSILs were attributable to 9vHPV vaccine types, accounting for 1.7% of vulvar LSILs, 16.1% of vulvar HSILs, 30.8% of vaginal LSILs, and 20.9% of vaginal HSILs (Table).

Conclusion

Widespread uptake of the 9vHPV vaccine could potentially prevent a sizeable fraction of benign and precancerous HPV-related vulvar and vaginal lesions.
Table. Proportionally weighted percentages of potentially vaccine-preventable HPV-positive vulvovaginal lesions.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>2vHPV</th>
<th>4vHPV</th>
<th>9vHPV</th>
<th>None&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted HPV genotypes&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16/18</td>
<td>6/11/16/18</td>
<td>6/11/16/18/31/33/45/52/58</td>
<td>35/39/51/56/59</td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>LSIL</td>
<td>18.8</td>
<td>25.2</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td>HSIL</td>
<td>53.1</td>
<td>57.4</td>
<td>78.3</td>
</tr>
<tr>
<td>Vulvar</td>
<td>LSIL</td>
<td>10.7</td>
<td>87.7</td>
<td>89.4</td>
</tr>
<tr>
<td></td>
<td>HSIL</td>
<td>74.3</td>
<td>83.9</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<sup>1</sup>Not covered by any of the 3 vaccines

<sup>2</sup>A total of 14 HPV types were assessed. Some lesions not categorized as HPV-related could have been caused by other HPV types for which genotyping was not performed. Some HPV types not genotyped could have been present in lesions categorized as HPV-related.
Background / Objectives

Cytotoxic T-cells (CTL) can mediate an anti-tumour response, but are 1) inhibited by regulatory T-cells (Tregs), 2) mislead by aberrant Human Leukocyte Antigen (HLA) expression on the tumour cells and 3) deactivated by Programmed Death Ligand 1 (PD-L1) on tumour cells or on tumour infiltrating macrophages (TIM). We aimed to gain insight in immunological factors in penile cancer, and their correlations with lymph node metastasis and disease specific survival.

Methods

Histological sections from a cohort of 213 penile cancer patients treated between 2000 and 2009 were used for immunohistochemical analysis. Human Papilloma Virus (HPV) status and different levels of HLA class I-expression were known from previous studies. Sections were stained for PD-L1, M2 macrophage-marker CD163, CTL-marker CD8, and Treg-marker FoxP3. PD-L1 was scored on TIM, tumour cells (percentage positive cells) and as tumour staining pattern (diffuse or predominantly at the tumour-stroma margin). Macrophages were scored as present/absent in tumour and stroma. The prognostic value of these parameters for lymph node metastasis (LN+ or LN-) and disease specific survival (DSS) was tested in univariable and multivariable regression models.

Results

Upon univariable analysis, strong intratumoural CD163 expression, non-classical HLA upregulation and diffuse tumour PD-L1 positivity were predictive for LN+, while high numbers of CD8+ T-cells in the stroma was associated with LN-. Regarding survival, HPV-positivity was significantly associated with improved DSS. Partial downregulation of classical HLA and a diffuse PD-L1 positivity in the tumour, with worse DSS.

Multivariable analysis determined HPV as a predictor for better DSS but not for lymph node status. Strong CD163 expression and a diffuse PD-L1 positivity in the tumour were both associated with LN+ and worse DSS. Other predictors included high CD8+ numbers in the stroma, associated with LN-, and partial downregulation of classical HLA, associated with worse survival.

When multivariably tested against clinicopathological predictors for lymph node status (lymphangioinvasion, grade of differentiation) and DSS (grade of differentiation, tumour size and lymph node status), HPV status (better DSS) and a
diffuse PD-L1 expression of the tumour (LN+ and worse DSS) were the only immune factors that remained significantly outcome correlated.

Conclusion

In penile cancer, various immune factors show correlations with lymph node status and ultimately, patient survival. These factors should be taken into account in future immunotherapy trials. The specific dissection of the tumour microenvironment characteristics will assist in patient tailored treatment.
Background / Objectives

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer. While the number of HNSCC cases is decreasing, the number of oropharyngeal SCC (OSCC) is rising. OSCC are increasingly (45-90%) caused by human papillomavirus type 16 (HPV16). Intriguingly, patients with HPV-induced OSCC (HPV16+) respond better to therapy than HPV-negative (HPV16-) OSCC. This is independent of nodal status, age, stage, tumor differentiation or gender. We hypothesize that tumors of HPV16+ OSCC patients display a different immune contexture that contributes to a better response of these tumors to standard therapy. To test our hypothesis, an exploratory in-depth analysis of immune infiltrates in the tumor microenvironment (TME) of 13 HPV16- and HPV16+ OSCC patients was performed using 36-parameter mass cytometry (CyTOF) analysis.

Methods

Tumor cell suspensions were prepared by mechanistic dissociation using GentleMac and cryopreserved until CyTOF analysis. HPV16 status of the tumors was determined by GP5+/6+ PCR and p16 immunohistochemistry staining. HPV16 E6/E7-specific T cell reactivity within HPV16+ tumors was determined by proliferation assay on directly ex vivo tumor samples and/or IL-2-expanded tumor infiltrating lymphocyte (TIL) batches. In-depth tumor immune infiltrate analysis was performed by CyTOF technology on ex vivo tumor samples from 13 OSCC patients, of which four were found to be HPV16-, four HPV16+ but HPV16 immune response-negative (HPV16+ IR-) and five HPV16+ immune response-positive (HPV16+ IR+).

Results

Analysis of the TME through 36-parameter CyTOF revealed clear phenotypic differences between immune cells infiltrating the TME of HPV16 IR+, HPV16+ IR- and HPV16- tumors. Whereas HPV16+ IR- tumors were strongly infiltrated with B cells, HPV16+ IR+ tumors were strongly infiltrated with effector memory CD4+ and CD8+ T cells with a highly activated, i.e. CD38+, HLA-DR+ and/or PD-1+, phenotype. Interestingly, subsequent unsupervised hierarchical clustering through the CITRUS algorithm led to the identification of two distinctive populations of activated CD4+ T cells and one population of CD103-expressing tissue-resident effector memory CD8+ T cells that were present at significantly higher levels in HPV16+ IR+ patients.
Conclusion

In conclusion, our data revealed that distinct immune cell populations infiltrate the TME of HPV16+ IR+ tumors that may contribute to a better response of these tumors to standard therapy.
ANTIBODY RESPONSE TO HUMAN PAPILLOMAVIRUS VACCINE (HPV) AMONG ALASKA NATIVE CHILDREN

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Background / Objectives

Routine vaccination with HPV vaccine is recommended at age 11 or 12 years. The 3-dose series can be started at age 9. We enrolled American Indian/Alaska Native (AI/AN) children into a study to determine response to human papillomavirus vaccine (4vHPV) post dose 1, 2 and 3, and duration of detectable antibody.

Methods

We recruited Alaska Native children aged 9-14 years from 2011-2014 who were vaccinated with 4vHPV. All children were tested one month post dose 3 and a subset was tested post doses 1 and 2. Antibody was measured using a multiplex L1-virus like particle-IgG ELISA.

Results

Among 470 children (400 girls and 70 boys) completing the 3-dose series, 432 (92%) were tested for antibody after dose 3, 71 (15%) post dose 1 and 70 (15%) after dose 2. Overall mean age at dose 1 was 11.2 years. All participants had detectable antibody after dose 3; after dose 1, 96-100% had detectable antibody. Geometric mean concentrations (GMCs) after dose 3 for HPV genotype 6, 11, 16, and 18 were 276.6 AU/ml, 355.6 AU/ml, 1256.4 IU/ml and 504.6 IU/ml, respectively. Among those tested after each dose, GMCs after dose 1, and 2 were: HPV genotype 6 3.8 AU/ml, 32.3 AU/ml; HPV11 5.4 AU/ml, 45.5 AU/ml; HPV16 20.9 IU/ml, 189.6 IU/ml; HPV18 6.1 IU/ml, 49.7 IU/ml. No serious adverse reactions were reported among vaccine recipients.

Conclusion

All Alaska Native children responded to vaccination. GMCs were higher after each vaccine dose. This cohort will be followed for a minimum of 20 years to determine duration of antibody response.
PRESENCE OF ANTIBODIES TO HPV IS HIGHLY CORRELATED WITH PRESENCE OF HPV DNA

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Background / Objectives

To determine the relation of the presence of HPV serum antibodies to presence of HPV DNA at the cervix in a large, longitudinal and population-based study with analysis of serial samples for specific antibodies to a large number of HPV types.

Methods

1848 women attending the organized national cervical cancer screening program in Slovenia on 2 different screening rounds 3 years apart were enrolled and tested for HPV DNA in cervical smears and for HPV specific antibodies (Abs) in serum, at both visits. Antibodies to HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 68 and 73 were determined using HPV pseudovirions. HPV DNA genotyping used Linear Array.

Results

Presence of HPV type-specific antibodies at any or both visits associated strongly with presence of HPV DNA of the same type. The association was present for all the oncogenic HPV types with an average OR for all the different types of 6.98 [95% CI 3.11-16.43]). Type specific Abs were mostly stable over time (65% of the initially seropositive women were positive at follow-up). Acquisition of antibodies from the first to the second visit was found in only 6% of baseline seronegative women and associated with HPV DNA detection at baseline (median p= 0.03) and for 5 of the HPV types also with self-reported new sexual partner.

Conclusion

A large-scale longitudinal study encompassing serology for 15 different HPV types finds that presence of HPV type specific serum antibodies associates strongly with presence of HPV DNA.
Seropositivity to multiple HPV types as a surrogate marker for current infection


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**Background / Objectives**

Antibodies to HPV are biomarkers for current and past infections. Our aim was to investigate the correlates of antibodies to multiple (≥3) HPV types.

**Methods**

2024 women participating in the organized cervical screening program in Slovenia were enrolled. They were tested for HPV DNA in cervical smears and for HPV antibodies in serum. 1848 of these women also attended a second round of screening 3 years later and had complete data on HPV antibodies and HPV DNA on both visits. Antibodies to HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 68 and 73 were determined using pseudovirion-luminex and HPV DNA typed with Linear Array. At baseline we also tested for antibodies to non-genital types (HPV 3, 5, 15, 32, 38 and 76). Women were stratified into three groups according to their HPV antibody status at baseline: seronegatives (N= 642), antibodies to 1-2 HPV types (N= 687) and ≥3 HPV types (N= 519). Associations between seropositivity and HPV DNA presence at different time-points/seropositivity to non-genital HPVs/association with cervical lesions were estimated with a chi square (χ²) test for linear trend.

**Results**

We found a strong positive trend between the baseline number of HPV types by serology and the baseline presence of HPV DNA in cervix (χ² = 68.8; p<0.0001). Baseline multiple seropositivity to HPV also strongly associated with baseline multiple HPV DNA positivity (χ²= 58.6; p<0.0001), with HPV DNA positivity at follow-up (χ²= 22.9; p<0.0001) and with baseline seropositivity to non-genital HPV types (χ²= 37.4; p<0.0001). Seropositivity to multiple HPV types also associated with presence of cervical lesions (χ²=9.8; p=0.0017). Baseline antibodies to multiple HPV types appeared to correlate with clearance as baseline multiple HPV antibodies was not significantly associated with multiple HPV DNA types at follow-up (χ²= 2.9; p<0.0866). Presence of antibodies to ≥3 HPVs tended to persist over time: 75% of these women still had multiple HPV type positivity 3 years later.

**Conclusion**
Seropositivity to at least 3 genital HPV types associates with current multiple infection and presence of cervical dysplasia.
SS 18-06
HLA CLASS II ANTIGEN EXPRESSION IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CANCER

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Background / Objectives

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. We characterized HLA class II antigen expression during HPV-induced cervical tumor development by examining the expression in CIN lesions and cervical cancers and correlated the results with immune cell infiltration.

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 epithelium adjacent to lesions (n=29). However, a strong and uniform staining pattern was found in the columnar epithelium and squamocolumnar junction zone. The percentage of HLA class II positive cells was low in CIN1 (40.9%), peaked in CIN2 (90.0%) and decreased 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 positive CIN1 lesions is compatible with the hypothesis that only a subset of CIN1, potentially those originating from the squamocolumnar junction zone, may overexpress HLA class II and tend to progress into high-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.
UNDERSTANDING TRANSCRIPTOMETRICS OF TOLL LIKE RECEPTOR (TLR) SIGNALING IN HPV-16 INFECTED CERVICAL CARCINOMA

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Background / Objectives

TLRs constitute important component of innate immune mechanisms1. HPV has been demonstrated to modulate TLR expression and signaling, leading to persistent viral infection and carcinogenesis2. This study was an attempt to understand the gene expression profile of TLRs, their downstream signaling molecules and other cancer initiating pathways triggered by them.

Methods

We performed HPV genotyping of the carcinoma and normal cervix using commercially available kits. Cases positive for HPV-16 were proceeded for expression profiling using PCR Array platform of TLRs (TLR 1-9), downstream TLR interacting molecules (MYD88, TICAM1, TICAM2, IRAK 4, IRF3, IRF7), molecules from cancer allied pathways namely AKT, NFκβ, MDM2, MTOR, p53, CDKN2A in HPV-16 infected, non-infected and carcinoma cervix. The protein expression of the relevant genes was studied at translational level using western blot.

Results

High risk HPV was detected in 23.1% (19/82) of normal cervical tissue. HPV16 (57.8%) was the preponderant subtype detected. 38/57 (66.6%) cases of SCC were HPV 16 positive. An overall down regulation in expression of all TLRs in carcinoma cases was observed with significant difference in TLR-4, TLR-6 and TLR-7 (p value 0.004, 0.050, 0.000). However, we observed highly significant upregulation of CDKN2A, AKT1 (p value 0.000, 0.038) in carcinoma tissue. At protein level, the expression of TLR7, TLR3 was found to be decreased with increase in expression of AKT, MDM2, CDKN2A confirming findings of gene expression.

Conclusion

The study demonstrates that HPV-16 plausibly targets innate immunity by significantly down regulating the expression of TLR-4, 6 and 7 in cervical cancer. In addition the altered expression of PI3K/AKT/MTOR axis and down regulation of p53 suggests involvement of viral oncogenes which are known to increase genomic instability, cellular proliferation and accumulation of mutagenic events resulting in cancer progression. Such events may probably be enhanced by increased
expression of MDM2 already identified to act as major negative regulator of tumor suppressor protein p53. Better understanding of the mechanisms employed by HPV for establishing persistence and carcinogenesis may assist in developing specific strategies for treating HPV related cancers.

References


Impact of vaccination on screening programs: what can we say today?

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Background / Objectives

Since 2006, when the first human papillomavirus (HPV) vaccine was approved, there has been much progress on the uptake of HPV vaccination in high and middle income countries. Australia, the first adopter of public, adolescent female HPV vaccination, coincidentally has had an organized program of cervical cancer screening for women 18-years of age and over, which permitted it to have an early surveillance mechanism to demonstrate an impact of vaccination in reducing the incidence of cervical precancerous lesions. As vaccination programs reach their 10th year anniversary, other countries have documented an impact in reducing the prevalence of cervical precancerous lesions associated with the vaccine-targeted HPV genotypes, replicating the findings from Australia. Another evidence of impact is the reduction in prevalence of vaccine-targeted genotypes in surveys conducted before and after the rollout of vaccination, which has been demonstrated in the US. In countries whose screening programs begin at or after age 25, 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 to risk. Few countries have begun pondering about the changes in screening that will have to be made, as well as the necessary information systems for surveillance, when a substantial proportion of women in the population have already been vaccinated.

Methods

N/A

Results

N/A

Conclusion

N/A
References

N/A
W 2-03
HPV-9 vaccine: all we need to know!

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Background / Objectives

A ninevalent HPV 6/11/16/18/31/33/45/52/58 vaccine was licensed in Europe in 2015 and has become available in 2016.

Methods

A review of the available data from the phase III trials and the current recommendations.

Results

The ninevalent vaccine has been evaluated in more than 27000 study participants. All these protocols and study results are published and available. The vaccine has been highly immunogenic in females and males from 9-26. The immunogenicity (and clinical efficacy) against HPV 6/11/16/18 is non-inferior to the quadrivalent HPV vaccine, the clinical efficacy against HPV 31/33/45/52/58 related disease and persistent infection is >96%. A 2dose schedule in girls/boys under the age of 15 has been is highly immunogenic and licensed by EMA and FDA. An administration in prior recipients of the quadrivalent vaccine is immunogenic and safe. The safety profile in general is favourable.

Conclusion

The ninevalent vaccine is licensed in Europe for males and females from the age of nine, under the age of 15 two doses with 6-12 months interval are sufficient. A study evaluating the immunogenicity in women up to the age of 45 is ongoing. The vaccine is safe, highly immunogenic in all evaluated populations and highly effective in preventing disease related to the nine HPV genotypes.

References

Review on HPV vaccine safety

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Background / Objectives

HPV vaccines are available for prophylactic use since a decade and, up to date, more than 180 million doses have been distributed worldwide. Vaccine uptake varies widely; some country programmes have been extremely successful, but vaccine hesitancy and obstacles to high acceptance of HPV vaccination is reported in many countries. The main obstacle to achieving high coverage levels is fear of alleged adverse events and rumours on HPV vaccine safety that easily spread across the globe.

Methods

Vaccine safety data collected during both pre-marketing and post-marketing phase have been critically reviewed and analysed.

Results

Pre-marketing studies on bi- tetra- and nona- valent vaccines have been carried out involving tens of thousands female and male subjects 9 to 26 years old. In addition, post-marketing safety evaluation have been carried out by both national and international public health agencies in order to assess the potential link between HPV vaccines and rare neurological syndromes. Most common adverse events following HPV vaccination are local reactions and some not serious general reaction linked to vaccine administration. No serious adverse event causally linked to HPV vaccines have been reported.

Conclusion

Safety asessement of all HPV vaccine products currently in use has been carefully carried out and the results are reassuring. HPV vaccines provide a very good safety profile also when co-administered to other adolescent vaccinations.

References


VACCINE TRUST AND HPV VACCINES: WHERE AND WHAT DO WE NEED TO DO?

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Background / Objectives

HPV vaccination programmes across the world are suffering from waving of public confidence and trust. In some countries - such as Japan, Denmark, or France - this has led to a sometimes dramatic decrease in the demand for the vaccine. Concerns have been shown to vary by region and country, but also by population group, with parents, teenagers, but also healthcare workers raising diverse concerns and doubts about the vaccine. Interventions developed with the aim of increasing HPV vaccination uptake and confidence should be based on a thorough understanding of the issues influencing public distrust.

Methods

The Vaccine Confidence Project has led various studies to broaden the understanding of factors and determinants associated to low public confidence in HPV vaccination. The results presented here primarily include those from a systematic literature review conducted in Europe, as well as an analysis of the media.

Conclusion

RESULTS/CONCLUSIONS: European populations were found to be particularly concerned about the safety of the HPV vaccine. In some cases, most commonly observed in online discussions, these concerns were related to rumours of specific side effects (POTS and CRPS). However, most safety concerns were reported to be broad and non-specific, and to rather reflect fears related to uncertainties and distrust. Beliefs that the vaccine is not effective, and that there is insufficient information available about the vaccine are among the uncertainties. Morality was another common issue, including perceptions that the vaccine could encourage promiscuity and unsafe sexual behaviours. Some countries are facing more challenges than others, calling for the development of context-specific interventions.

Communicating scientific facts is not sufficient, especially in a context of post-truth societies. Interventions to address HPV vaccination confidence should be informed by an understanding of key issues that influence public trust and distrust. Other possible interventions that encourage dialogue include the organisation of peer-to-peer information and discussion sessions, or through healthcare worker-moderated online discussion groups.
WHAT IS NEW WITH THE VULVAR TERMINOLOGY?

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Background / Objectives

The approach to vulvar disease, which has been changed lately, has led to the introduction of the new terminologies for vulvar conditions: The IFCPC clinical and colposcopic terminology, The ISSVD terminology of Vulvar Squamous Intraepithelial Lesions (Table 1) and the consensus terminology of vulvar pain and vulvodynia (Tables 2 and 3).

Methods

Table 1: 2015 ISSVD Terminology of Vulvar Squamous Intraepithelial Lesions

Low grade squamous intraepithelial lesion of the vulva [Vulvar LSIL]
High grade squamous intraepithelial lesion of the vulva [Vulvar HSIL]
Vulvar Intraepithelial neoplasia [VIN], differentiated-type [DVIN]

Results

Table 2: 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 (e.g. vestibulodynia, clitorodynia) or Generalized or Mixed (localized and generalized)
· Provoked (e.g. insertional, contact) 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

Conclusion

Table 3: 2015 Consensus terminology and classification of persistent vulvar pain and vulvodynia

Appendix: Potential factors associated with Vulvodynia*

- Co-morbidities and other pain syndromes (e.g. painful bladder syndrome, fibromyalgia, irritable bowel syndrome, temporomandibular disorder) [LOE 2]
- Genetics [LOE 2]
- Hormonal factors (e.g. pharmacologically induced) [LOE 2]
- Inflammation [LOE 2]
- Musculoskeletal (e.g. pelvic muscle overactivity, myofascial, biomechanical) [LOE 2]
- Neurologic mechanisms:
  - Central (spine, brain) [LOE 2]
  - Peripheral – Neuroproliferation [LOE 2]
  - Psychosocial factors (e.g. mood, interpersonal, coping, role, sexual function) [LOE 2]
  - Structural defects (e.g. perineal descent) [LOE 3]

*The factors are ranked by alphabetical order
LOE - Level of evidence
HOW TO PERFORM HIGH RESOLUTION ANOSCOPY (HRA)

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Background / Objectives

The incidence of anal cancer in the general population is higher among women than men, and has been rising steadily since the 1970s. Although the incidence in the general population is relatively low, certain groups of women are at increased risk of anal cancer. These include women with a history of cervical or vulvar high-grade squamous intraepithelial lesions (HSIL) or cancer and women with immunocompromise due to HIV infection or other causes such as medication to prevent transplant rejection. As with algorithms designed to identify and treat cervical HSIL to prevent cervical cancer, anal cancer prevention programs include visual inspection of the at-risk areas. For anal cancer prevention this technique is known as high resolution anoscopy (HRA).

Methods

HRA includes the use of a colposcope to identify anal HSIL, identify areas to with biopsy to determine the grade of disease and ultimately guide targeted removal of lesions to reduce the risk of cancer.

Results

Although there are many similarities between colposcopy and HRA, including the use of acetic acid and Lugol’s solution, there are also several differences. These include the need to maintain focus over a wider length of at-risk mucosa; the need to focus the colposcope through an internal plastic anoscope; the need to manipulate the anoscope and swabs/brushes to flatten folds and push aside other normal anatomic features such as hypertrophic papillae to properly visualize the entire mucosa; and the need to remove blood, mucus and stool. Other common and/or normal anatomic features may pose challenges to a complete HRA including anal crypts and hemorrhoids. In addition while acetic acid and Lugol’s solution have been validated to identify anal HSIL, patterns of vascular change and other signs of anal HSIL differ somewhat from those of the cervix. A complete HRA includes inspection of the entire squamocolumnar junction (SCJ), the area proximal to the SCJ, the mid-canal to the dentate line, the distal canal to the verge and the perianal keratinized skin to a radius of 5 cm from the anal opening. The appearance of anal lesions varies considerably throughout this wide range of epithelial surface. The performance of acetic acid and Lugol’s solution to identify anal HSIL varies as well, as do the challenges associated with identifying disease at these different locations.

Conclusion
HRA is an important tool to identify anal HSIL, biopsy it to confirm the grade of disease and to treat it to prevent progression to anal cancer. Gynecologists should consider learning and performing this technique in women at increased risk for anal cancer.
W 4-03
A new era of DNA Immunotherapy for Vulvar HSIL

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Background / Objectives

Vulvar HSIL remains a condition with no licensed alternatives to surgery. The recurrence rate under current surgical and medical approaches is extremely high. There is a high psychological and physical negative impact of surgery. Vulvar HSIL is symptomatic and impacts activities of daily living. Prophylactic HPV vaccines have no therapeutic activity against vulvar HSIL and have not achieved universal uptake, leaving a population of susceptible and impacted women. The early diagnosis of vulvar HSIL is limited to visual inspection with biopsy. We now present the final data for a Phase 2b study showing proof of concept against cervical HSIL of VGX-3100 an investigational DNA-based immunotherapy and present our trial design approach to studying efficacy against VIN.

Methods

For the cervical HSIL study 169 subjects were randomized: 127 subjects in the VGX-3100+EP arm and 42 in the placebo+EP arm. Female subjects aged 18 to 55 years with histologically confirmed HPV-16 or HPV-18-associated high grade CIN from tissue collected less than 10 weeks prior to the first injection/EP with no evidence of invasive cancer in any specimen were eligible. One mL of VGX-3100 (designed to enable the immunologic targeting of HPV-16 E6 and E7, and HPV-18 E6 and E7) was administered IM, followed by EP using the CELLECTRA 2000 EP system three times during the study (Day 0, Week 4, and Week 12).

Results

The study met its primary efficacy endpoint; treatment with VGX-3100 significantly improved histologic regression of CIN2/3 to CIN1 or normal pathology during the Week 36 primary analysis period. In the mITT population, histopathological regression occurred in 48% (55/114) of the VGX-3100 recipients compared with 30% (12/40) of the placebo recipients (strata adjusted percentage point difference, 17.8; 95% CI, 1.3-34.4; p=0.034). The secondary endpoint of concomitant histopathological regression to CIN1 or normal pathology and clearance of HPV-16 or HPV-18, or both HPV-16 and HPV-18 (virologic clearance) during the Week 36 primary analysis period after immunization with VGX-3100 was also met. In the mITT population, concomitant histopathological regression and virologic clearance occurred in 41% (46/112) of the VGX-3100 recipients and 15% (6/40) of the placebo recipients (strata adjusted percentage point difference, 23.9; 95% CI, 9.3–38.5; p=0.001). mITT results were similar to PP.

Conclusion
Proof of concept was demonstrated for VGX-3100 against HPV-16 and HPV-18 associated cervical HSIL. We now propose an approach to study VGX-3100 as a treatment for HPV-16 and/or HPV-18 associated vulvar HSIL, which is an area of significant undermet medical need.

References

Background / Objectives

The first descriptions of a disorder very similar to what we nowadays define as vulvodynia can already be found in the literature in the late 19th century. Since then, the classification and terminology have undergone remarkable changes. The most recent update occurred in April 2015 at a consensus conference of the International Society for the Study of Vulvovaginal Disease (ISSVD) and two other societies, the International Pelvic Pain Society (IPPS) and the International Society for the Study of Women's Sexual Health (ISSWSH). This 2015 classification defines vulvodynia as vulvar pain of at least 3 months duration, without clear identifiable cause, which may have potential associated factors. Further, vulvodynia is characterized as either localized (affecting only a part of the vulvar area, e.g., the vestibule or clitoris) or generalized (present in the whole vulvar area). Vulvodynia may be provoked (sexual, nonsexual, or both), spontaneous, or mixed, and the onset can be primary (present at the first attempted introital penetration) or secondary (appearing later in life after a period of painless intercourse). Vestibulodynia refers to the pain in the vulvar vestibule (i.e., the area surrounding the vaginal opening). The term vestibulodynia was recommended for the first time in 2003 by the ISSVD when it replaced the previous term "vulvar vestibulitis syndrome" (VVS). The terms localized provoked vulvodynia (LPV) and localized provoked vestibulodynia (LPV) are both widely used, and the condition may also be called as provoked vestibulodynia (PVD).
Localized provoked vulvodynia: pathogenesis and pain mechanisms

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Background / Objectives

Localized provoked vulvodynia (LPV) is a subset of vulvodynia, associated with induced pain by touch on vulvar mucosa in the absence of any other recognizable disease. The intensity of pain is out of proportion to the applied pressure: very light touch evokes excessively strong pain, a phenomenon defined as allodynia. Different vulvovaginal infections, especially recurrent candidiasis, and urinary tract infections are known risk factors for LPV. LPV patients, more often than healthy controls, are genetically predisposed to yeast and other vulvo-vaginal infections. Animal models and in vitro studies have further produced data of the significance of Candida albicans infection as a pain generator in the vulvar vestibule. Studies conducted in cell cultures have shown that fibroblasts originating from vestibular mucosa of LPV patients are more responsive to fungal antigens than those from healthy controls. LPV patients also have a tendency to carry pro-inflammatory allele variants of IL-1beta and IL-1 receptor antagonist genes and show elevated tissue levels of pro-inflammatory cytokines. An increased immunoinflammatory response and the neuroinflammatory axis may well be involved in the development of a chronic pain syndrome.

Methods

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Results

Recently we demonstrated the existence of secondary lymphoid tissue, the vestibule associated lymphoid tissue (VALT) in vestibular mucosa and showed that this VALT had become activated in LPV. In VALT, like in other mucosa-associated lymphoid tissues (MALT), antigen presenting dendritic cells transport foreign antigens to germinal centers where the initiation of an immune response (B and T cell activation) takes place. 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 such areas of the mucosa and vestibular glands, which showed increased B lymphocyte infiltration. Also, the density and presence of IENFs were higher in samples with more pronounced immune activation.

Conclusion
This further supports the essential role of immune activation in the altered pain sensation of LPV. Interplay between activated immune cells and biomodulators of the signaling of sensory neurons could thus be involved in LPV.

References

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CONSERVATIVE MANAGEMENT OF VULVA PAIN SYNDROME

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Background / Objectives

Vulvar pain affects women at all ages. It may be due the complexity of the clinical presentation and pathophysiology involved in vulvar pain due to a specific disorder or diagnosed as vulvodynia. Vulvodynia is defined as vulvar pain of at least 3 months' duration, without clear identifiable cause, which may have potential associated factors. The etiology of vulvodynia is still not exactly known, however many associated factors are acknowledged. These factors are among others: other pain syndromes (e.g., painful bladder syndrome, fibromyalgia, irritable bowel syndrome, temporomandibular disorder), Genetics, Hormonal factors (e.g., pharmacologically induced), Inflammatory, musculoskeletal (e.g., pelvic muscle overactivity, myofascial, biomechanical), Neurological, central and peripheral nervous system affection, Psychosocial factors (e.g., mood, interpersonal, coping, role, sexual function), Structural defects (e.g., perineal descent).

Methods

The purpose of the presentation is to share knowledge on the various conservative treatments and the outcome on vulvar pain and vulvodynia.

Results

Results from several studies all demonstrate that women with vulvodynia are significantly affected on all domains of their sexuality and report significantly higher levels of sexual distress compared to controls. Women with vulvar pain are also affected on dyadic parameters and psychological wellbeing. These results among many others imply that clinicians need to address the sexual functioning and level of sexual distress in women diagnosed with vulvodynia, and to consider psychosexual treatment as part of a multidisciplinary treatment program to reduce pain in the vulva.
Conclusion

As recommended by the International Society for the Study of Vulvovaginal Disease, International Society for the Study of Women's Sexual Health, and International Pelvic Pain Society, treatment on vulvar pain should be chosen according to the characteristics of the individual case and the possible associated factors, rather than as a “one-size-fits-all” approach. A multidisciplinary treatment approach is therefore recommended including conservative treatment.

References


3. Thoñner LB et al. Multidisciplinary treatment on vulvodynia--where is the evidence? Ugeskr Laeger. 2014 Dec 22;176(52)
SURGICAL MANAGEMENT OF VULVAR VESTIBULITIS SYNDROME BY VESTIBULECTOMY

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Background / Objectives

Vulvar vestibulitis syndrome (VVS), also called vestibulodynia and localised provoked vulvodynia (LPV), a subset of vulvodynia, is a complex vulvar pain syndrome (VPS) characterised by altered pain sensation. VVS causes severe dyspareunia and affects mainly young women. Recent studies on VVS have demonstrated activation of vestibule-associated lymphoid tissue (VALT) which may emerge as a response to localised inflammation. Immune activation enhances epithelial nerve growth in the vestibular mucosa causing allodynia. No uniformly effective treatment exists. Clinical algorithms have been developed to augment the management of VVS.

Methods

Methods and results: Surgical treatment is usually offered to the most severe cases refractory to conservative treatment modalities, including discontinuation of oral contraceptives, physical therapy for pelvic floor dysfunction, and antimycotic maintenance therapy. Vestibulectomy operation is strikingly effective in severe cases, and has high patient satisfaction. Vestibulectomy is a day surgery operation with low risk for postoperative complications. Recurrence rate is low. In the operation, vestibular mucosa is removed, and replaced by properly liberated vaginal mucosa.

Conclusion

One problem is the lack of randomised controlled trials of vestibulectomy. This is not surprising, since vestibulectomy is generally offered as the last resort in the most severe refractory cases. Long-term follow-up studies have demonstrated that this operation has favorable outcome and significantly improves the quality of life of women with severe VPS.
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SOCIO-ECONOMIC AND DEMOGRAPHIC DETERMINANTS OF PARTICIPATION IN THE SWEDISH CERVICAL SCREENING PROGRAM: A POPULATION-BASED CASE-CONTROL STUDY

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Background / Objectives

Identify socio-economic and demographic determinants for non-attendance in cervical screening

Methods

Design: Population-based case-control study

Setting: Sweden

Population: Source population was all women eligible for screening. Based on complete screening records, two groups of women aged 30 - 60 were compared. One group (N=266,706) attended within 90 days of invitation. The other group (N=314,302) had no smear registered for 6-8 years.

Main outcome measures: Risk of non-attendance by 9 groups of socioeconomic and demographic variables

Methods: Unadjusted odds ratios (OR) and OR after adjustment for all variables in logistic regression models were calculated.

Results

Women with low disposable family income (OR 2.06; 95% confidence interval (CI) 2.01-2.11), with low education (OR 1.77; CI 1.73-1.81) and not cohabiting (OR 1.47; CI 1.45-1.50) were less likely to attend cervical screening. Other important factors for non-attendance were being outside the labour force and receiving welfare benefits.
Swedish counties are responsible for running screening programs; adjusted OR for non-participation in counties ranged from OR 4.21 (CI 4.06-4.35) to OR 0.54 (CI 0.52-0.57), compared to the reference county. Being born outside Sweden was a risk factor for non-attendance in the unadjusted analysis but this disappeared in certain large groups after adjustment for socioeconomic factors.

Conclusion

Low income and low education were associated with increased probability of non-attendance. Low attendance among large groups of immigrant women might be explained by socio-economic factors. Residing in particular Swedish counties was also a strong independent factor. As counties are responsible for effectuating the screening program this indicates considerable potential for improvement of cervical screening attendance in several areas if best practice of routines is adopted.
SCREENING HISTORY IN CERVICAL CANCER PATIENTS ≥ 55 YEARS DIAGNOSED DURING 1990-2013 IN DENMARK

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Background / Objectives

The incidence of cervical cancer has declined significantly in developed countries following the implementation of cervical cancer screening. However, previous studies have reported that ~50% of cervical cancer patients have not attended screening and that the fraction of unscreened women increases by age. This study aimed to describe the temporal pattern of screening in cervical cancer patients ≥55 years diagnosed during 1990 – 2013 at Aarhus University Hospital, Denmark

Methods

This hospital based cohort study included women ≥ 55 years diagnosed with cervical cancer at the Department of Pathology, Aarhus University Hospital, Denmark during 1990-2013 (n=515). Information on their previous history of cervical cancer screening was obtained from the Danish Pathology Databank.

Results

Overall, 47.0% (95% CI 42.6 – 51.4) had never been screened prior to cervical cancer diagnosis. The fraction of never screened cases declined over calendar time from 69.8% (95% CI 61.4 – 77.3) in 1990 – 1994 to 20.0% (95% CI 12.7 – 29.2) in 2010 – 2013, reflecting a period effect of screening. Conversely, the fraction of never screened cases increased by age from 22.5% (95% CI 14.6 – 32.0) in women aged 55 – 59 years to 63.2% (95% CI 49.3 – 75.6) in women ≥ 80 years. Noteworthy, the vast majority of never screened (90.9% ; 95% CI 86.6 – 94.2) lived in a period where screening was not available, whereas only 9.1% (95% CI 5.8 – 13.4) had not been screened although screening was available. Among women who had been screened 5 years or 5-10 years prior to cervical cancer diagnosis, 84.6% (95% CI 77.1 – 92.2) and 85.9% (95% CI 75.0 – 93.4) had a normal cytology result, respectively.

Conclusion

Cervical cancer in older women may partly be attributed to a lack of screening or due to a failure in screening. However, older women were in general less screened because they either lived in a period where screening was not available or they were too old to be screened when screening was implemented. Furthermore, since
previous studies have shown that older women are at high risk of cervical cancer\textsuperscript{1,2}, more information on how to best screen older women is needed.

References


2. Increased age and race-specific incidence of cervical cancer after correction for hysterectomy prevalence in the United States from 2000 to 2009.

Rositch AF, Nowak RG, Gravitt PE. Cancer. 2014 Jul 1;120(13):2032-8.
INVITING WOMEN TO CERVICAL CANCER SCREENING AT THE AGE OF 65

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Background / Objectives

In Finland the organized cervical cancer screening program invites women for routine screening up to the age of 60. Some municipalities also invite 65-year-olds. The aim is to study whether screening at the age of 65 reduces cervical cancer mortality.

Methods

Screening records for women aged 55 and above were collected from the mass screening registry in 1991—2014 (612 622 women born in 1926—1946). Cervical cancer deaths (N=265) were linked from the cancer registry for women aged 65 and above.

Results

Of all women, 383 411 were invited of whom 85% attended to screening at least once during the follow-up. Of all invited women, 77 479 (13%) received an invitation to routine screening at the age of 65. The risk of death due to cervical cancer was reduced for women who were screened at the age of 65 (RR = 0.57 (95% CI = 0.32−0.95)).

Conclusion

Mortality was reduced for women screened at the age of 65. However, a more detailed examination of the effect of previous screening history is still needed, e.g. taking into account participation to screening at younger ages and previously detected abnormalities. The results will help to assess until what age the whole target population should be invited to screening.
EVALUATION OF THE CERVICAL CANCER SCREENING PROGRAM IN THE FLEMISH REGION BY THE BELGIAN CANCER REGISTRY

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Background / Objectives

An organised population-based cervical cancer screening was set up in 2013 in the Flemish Region for women aged 25 to 64, based on a call-recall system. Cytology is used as primary screening test and HPV detection as triage for atypical cells. The Belgian Cancer Registry (BCR) calculates yearly quality indicators to monitor this program on demand of the Agency for Care and Health of the Flemish Ministry of Welfare, Public Health and Family.

Methods

Besides new cancer diagnoses, the BCR collects all anatomo-pathological results of cervical samples in a central cyto-histopathology registry, which is completed with administrative data from health insurance companies. BCR plays a crucial role in the cost-effective organisation and the quality assurance of the screening program due to the centralisation of all these data and due to the possibility of linking at the personal level using a unique patient identifier. By linking these databases with a Flemish population registry, BCR calculated for 2013 several quality indicators.

Results

In 2013, 64% of the Flemish female population between 25 and 64 years old was covered by the screening program. 7% of the eligible women had an abnormal screening. 27% of the women with an abnormal screening had no follow-up within one year. 236 new invasive tumours were diagnosed within the target population. Analysis of the screening history revealed that 111 of these tumours were diagnosed in women that were not screened within 5 years before. About 40% of the tumours in these non-screened women are stage I. In contrast, more than 70% of the women who had at least one screening in the last 5 years had a stage I tumour. 82 of the 236 women with an invasive tumour were tested for HPV in the past 5 year, whereof 10 with a negative HPV test result.

Conclusion

Quality indicators reveal the weaknesses in the screening program. They can be directly translated into policy decisions to increase the program coverage, to improve the follow-up rate after abnormal screening and to investigate whether or not these...
cancers are truly HPV negative. Centralisation of databases and the possibility of individual linking are crucial to a successful screening program.

References

Acknowledgements

The Agency for Care and Health, part of the Flemish Ministry of Welfare, Public Health and Family, finances the Flemish cervical cancer screening program. It subsidizes the Center for Cancer Detection to carry out the program and the BCR to support the organization and evaluation of the program.
Cervical Screening in Sweden in 2015

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Background / Objectives

To collect data to enable calculation of key quality indicators and basic statistics on cervical screening in Sweden.

Methods

We collected individual level data on all (both organized and non-organized) cervical cytologies and histopathologies in Sweden in 2015, as well as individual level data on all invitations for cervical screening that were sent.

Results

There were 723,500 cervical smears (662,350 tests were cytologies and the remainder were HPV tests). Organized smears (smears resulting from an invitation) constituted 69% of smears. The screening test coverage of the target population was calculated by linkage with the population registry (all resident women aged 23–60) and found to be 81%. The coverage has been similar for >10 years, but varied greatly between counties (from 71% to 91%) and over time. The incidence of the disease screened for (cervical cancer) was also stable over time at the national level, but varied between counties. There were 7,982 women with HSIL+/AIS in cytology in 2013. Of these, 181 women had not been followed up with biopsy by 2014-12-31.

Conclusion

Straightforward collection of all screening data in the country enables reliable reporting of screening quality indicators which are the basis for evidence-based optimization and innovation of the program.
FC 01-06
NORDSCREEN – AN INTERACTIVE TOOL FOR PRESENTING CERVICAL CANCER SCREENING INDICATORS IN NORDIC COUNTRIES

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Background / Objectives

Quality assurance and improvement of cancer screening programs require up-to-date monitoring systems and evidence-based indicators. The Nordscreen database [1] is planned to include performance and outcome indicators for cervical cancer screening programs in the Nordic countries and Estonia. The tool will be publicly available and facilitate comparison of cancer screening programs over time and between the Nordic countries.

Methods

The screening data originates from population-based mass screening registries in each of the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) and Estonia. Comparability between countries is ensured by using uniform data structure. The developed indicators are based on the European guidelines for cervical cancer screening and other ongoing research projects. [2] Fact sheets summarising the cancer screening policies and programs in place in all the Nordic countries and Estonia will be created to provide context for the indicators.

Results

Currently cervical cancer screening test coverage data is available from Norway (years 1992 - 2015) and Finland (1991 - 2014) with other Nordic countries and Estonia to be included soon. The test coverage within screening interval of 3 years in age group 30-59 was 75.0% in Norway in 2014 (60.4% in Finland). Test coverage in 2014 increased to 90.4% in 10-year follow-up in Norway (82.2% in Finland). The application can be present data in graphical or table form based on user specifications such as follow-up time, calendar year and age group.

Conclusion

Lower test coverage in Finland can be explained by policy of 5-year screening interval and that mass screening registry in Finland only includes tests that are provided within the organized screening process whereas Norwegian data also includes opportunistic screening tests. Despite some limitations, the performance and outcome indicators are likely to be relevant to many stakeholders such as researchers, policy makers and journalists. In the future, the database may also be expanded to on-going screening programs for breast cancer and colorectal cancer.
References


TEN YEARS EXPERIENCE IN 541.000 CASES: LIQUID BASED CYTOLOGY AND COMPUTER-ASSISTANCE COMPARED TO CONVENTIONAL CYTOLOGY

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Background / Objectives

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 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 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 split this analysis between privately insured and self-paying 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 unofficial 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 2016 463.966 slides of privately insured patients have been analyzed among them 320.416 by TIS and 143.550 with CC. Except of extremely bloody and very cell-rich probes 97.4% of the smears were accepted for analysis by TIS. TIS had a rate of LSIL (low grade intraepithelial lesions; MN III: Pap IIID1) of 2.03% compared to 0.54% for CC, an increase of 276%. HSIL (MN III: Pap IIID2 + Pap IVa/b) was found in 1.10% with TIS vs 0.33% with CC (+233%). The ASC-US rate (MN III: Pap II-p/g + III-p/g) was 2.54% with TIS and 1.21% with CC, an increase of 110% which is much lower than the rise in LSIL and HSIL. This points to a higher sensitivity of TIS without decreasing specificity. Among 77.282 self-paying patient cases (all TIS) we found almost the same rates for ASC-US and LSIL but 51% more for HSIL compared to private patients. All these results remained stable over the 10 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.
The value of “diagnostic cytology” with p16/Ki-67 dual-staining

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Background / Objectives

When implemented appropriately, cervical cancer screening has the power to save lives. Its effectiveness, however, rests on the sensitivity and specificity of the diagnostics themselves—namely, pap cytology and HPV DNA testing. The introduction of p16/Ki-67 dual-stain cytology as a triage test for abnormal pap results offers the ability to improve detection of precancer in equivocal and abnormal cervical cells. This study seeks to evaluate the performance of dual-stain “diagnostic cytology” in Belgium (women age 25-65) through a systematic literature review; this includes a component of meta-analysis in order to draw conclusions about the increasingly complex, quantitative body of existing knowledge available.

Methods

A literature search was conducted of published studies from 1 May 2016 according to CRD, PRISMA, and NICE guidelines. Studies needed to report diagnostic performance with key metrics: sensitivity, specificity, detection rate, odds ratio, PPV, NPV, true and false positives, and true and false negatives. These outcomes were chosen since they are the most significant and widely used outcomes to compare performance, and can be calculated from one another using established formulas. Sensitivity and specificity were chosen as outcome measures in the final meta-analysis based on guidance from the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. The meta-analysis of dual-staining performance focused on sensitivity and specificity. Results of multiple studies collected during the literature review were combined into a single, standardized metric for comparison across different tests.

Unlike meta-analyses of treatment intervention effects, meta-analyses of diagnostic test accuracy must allow for the trade-off between sensitivity and specificity that occurs between studies whose threshold values for test positives and negatives vary. Hierarchical models (in contrast to many classical regression models) allow for correlation between sensitivity and specificity, in addition to the aforementioned trade-off. Therefore, the bivariate random effects model was used in this meta-analysis because of its ability to jointly evaluate sensitivity and specificity, thus providing an estimate of the diagnostic accuracy of dual-staining.

Conclusion
Dual-stain “diagnostic cytology” with p16/Ki-67 is an attractive biomarker for triage in cervical cancer screening. In a Belgian screening population (age 25-65 years), dual-stain cytology offered significant gains in sensitivity with minimal reduction in specificity. This could lower the number of cases of missed disease and reduce morbidity from additional interventions such as colposcopy and biopsy.
Background / Objectives

The Swedish cervical screening program is changing from cytology to HPV based screening in 2017. To document the performance of the screening program and to provide a basis for evaluating the effect of changes in the program we performed a nationwide audit of the cervical cancer cases from 2002 to 2011. Our aim with this analysis is to compare different sources of information for the tumor histological classification, and to determine how robust this classification is for subsequent analyses of data.

Methods

Data on all 4254 cases of cervical cancer or unspecified uterine cancer diagnosed between 2002 and 2011 was identified from the Swedish National Cancer Registry. Tumor histopathology is provided as a SNOMED classification code by the local pathologist and the clinician at the initial diagnosis. For the Audit an experienced gynecologist reviewed all medical records to identify cervical cancer cases with primary, invasive, epithelial tumors of cervical origin and to extract relevant data on e.g. treatment, mode of detection and histological classification of the tumor. Additionally we collected diagnostic slides and tissue blocks: an external review of the tumor was performed by a senior pathologist and HPV-genotyping was done on the tissue material. The Swedish National Cervical Screening Registry (NKCx) provided data on screening for all cases and matched population controls.

Results

One of 26 different SNOMED codes was reported for all except 6 cases. The systematic external review on 86% of the cases classified the tumors into following groups: Squamous Cell Carcinoma, Adenocarcinoma, Adenosquamous carcinoma and rare histological types (i.e. neuroendocrine, small cell, undifferentiated cancer). The medical record provides information on histology in various levels of detail and in a non-systematic form, so we could only extract this information for 69% of cases, in the same categories as above. There is a high concordance (87%) between all three sources of histology classification. For 9.8% of cases with discrepancies in the histology classification or missing data we decided for a final classification based on:
1) external histopathology review of the sample (in cases of good quality samples), 2) medical record and 3) initial SNOMED diagnosis (this was changed only in 7.5% of cases) for subsequent analyses of data.

Conclusion

Thorough nationwide ascertainment from multiple sources found limited ambiguity regarding tumor histology. For most uses, any one of the sources can be used, as the concordance between different sources of data is high.
HPV testing in routine cervical screening in rural Malawi – prevalence, link to clinical findings and challenges

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Background / Objectives

Developed countries are moving fast to replace cytology-based cervical screening with HPV primary testing. Our primary objective was to establish feasibility of HPV testing for primary screening in Malawi and to identify/address the major challenges to implementation, including outcomes of different collection devices and media. We also aimed to determine prevalence of HPV genotypes in the Nkhoma region.

Methods

Specimens were obtained from women attending routine VIA (Visual Inspection with Acetic acid) clinics in Nkhoma Hospital catchment area. VIA assessments were carried out by competent providers. HR-HPV prevalence was established using samples collected in Preservcyt® and tested by Xpert®HPV according to manufacturer’s instructions. Modifications were also tried, including reduction in collection volume, change of collection medium, use of self-collected samples and different collection devices. A sensitive multiplex PCR based assay (Papilloplex AnyHPV) was used for genotyping on a subset of samples selected according to the VIA result (139 VIA -; 156 VIA+; 42 VIA suspicious cancer).

Results

HR-HPV positivity using Xpert HPV was ~20%(n=750). Multiple infections were common and HR-HPV prevalence in HIV+ women was 43%. For HR-HPV, concordance was good between Xpert and Papillolplex HPV tests (k=0.68). In women with suspicious cancers HPV16,18 and 45 predominated (22.7%, 11.4% and 11.4%). The most frequently detected HPV type in VIA+ women was HPV16 but Xpert P3 group (HPV52>35>31>33>58) dominated. A number of VIA+ women were HPV negative, most were not due to LR-HPV presence. HPV+ results were frequently reported in VIA- women, with HPV16 the most frequent individual type, while P3 predominated and LR-HPV was detected in ~20%. Reduction of collection medium to 5ml, alternative media and self-collected samples gave comparable HPV results.

Conclusion
HPV16 was the commonest individual type detected in women who are VIA+ /suspicious cancer and in VIA- women. HPV31 and related types (Xpert P3) is the most commonly detected subgroup in VIA+ and VIA- women but not in those with suspicious cancers.

Xpert HPV showed high concordance with Papilloplex for HR-HPV. The latter test is more sensitive and detected LR-HPV, but is less suitable for LMIC4. Xpert® HPV is straightforward, has rapid turnaround and should be validated with low cost collection systems.

There was high correlation between VIA suspicious cancers and HR-HPV positivity but disagreement between HPV positives and VIA positives requires further analysis before considering HPV testing for primary screening.

References

REFERENCES

Kawonga E, Mwenitete I, Mautanga M et al. Poster#143, HPV2017, Cape Town, March 2017
Bhatia R, Kawonga E, Mhango E et al. Abstract # EUROGIN 2017, this meeting

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Cervical cancer screening in the remote island of Principe

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Background / Objectives

The island of Principe, part of the archipelago of São Tomé and Príncipe, in the Gulf of Guinea, suffers the problems inherent to its double insularism. Its 7,000 inhabitants have very limited access to health care. In 2014, the privately funded NGO Ascendere started a cervical cancer screening program. A previous report has shown that this island has a unique HPV genotype distribution: HPV prevalence is high (36.7%), but HPV16 and 18 are very rare (0 and 2%, respectively) 1.

Methods

Retrospective evaluation of the records of the women screened between January 2014 and September 2016.

Results

During this time period 972 women were screened, with a mean age of 35.5±10.04 years old (21-83 years old). Out of these, 968 (99.6%) had a satisfactory Pap test; 150 (15.5%) had an abnormal Pap test: ASC-US 58 (6.0%), LSIL 56 (5.8%), LSIL-H 2 (0.2%), ASC-H 9 (0.9%), HSIL 23 (2.4%), carcinoma/adenocarcinoma 2 (0.2%). Colposcopy was indicated in 111 (11.4%) women; 31 (27.9%) of them did not show up for evaluation. An histologic diagnosis of HSIL (CIN2 p16+/CIN3) was made in 35 women (3.6%) and of invasive carcinoma in 2 (0.2%). Of the women with HSIL, 33 (94.3%) had an excision of the transformation zone.

The mean age of women with a final histological diagnosis of HSIL/invasion was similar to those without evidence of it (36.5±11.59 vs. 35.5±9.97 years, p=0.542). Only one case was detected in a woman younger than 25 years old. There were no differences in contraception use, parity, use of alcohol, menopause status, existence of co-infections (C. trachomatis, N. gonorrhoea and T. vaginalis), age of sexual debut, menarche, age of first delivery, or number of sexual partners in women with and without HSIL/invasion. There were no differences in HIV status or smoking, but the occurrence of both risk factors is very rare in this setting.

Conclusion
Strategies must be implemented to reduce the number of women lost to follow-up. Despite the low prevalence of HPV16 and 18, still there is an elevated risk of HSIL, which justifies the maintenance of a cervical cancer screening program and the introduction of the nonavalent vaccine.

References

Background / Objectives

The incidence of cervical cancer (CC) varies greatly with a large difference between developing and developed countries, where CC cases have been significantly reduced since the implementation of effective screening programmes. 1. Exfoliative cervicovaginal cytology has been regarded as the gold standard for cervical cancer screening programs limitations are Incorrect and inadequate sampling in 5-10% of cases. 2. Manual Liquid Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer and thus improves detection of precursor lesions and specimen adequacy. the residual sample can be used for other tests like detection of HPV, DNA and immunocytochemistry. Cell blocks can be prepared from all types of cytological specimens, with advantage of cell blocks is that many slides can be prepared for extensive panels of immunostains. 4. Overexpression of p16INK4a in almost all cervical precancer has been shown to be directly linked to the transforming activity of E7 oncoprotein, which is produced by HPV. Cellular accumulation of p16INK4a 1. Our current PCR set-up gives rapid, type-specific HPV detection with a turnaround time of less than 24 hrs and cost-effectiveness compare to commercial available alternatives. 4,5

Methods

Samples from examined patients were collected using the direct to vial technique from 75 patients in the age group of 20 to 60. cervix brush was used to scrape the cervix. were done for collecting samples for hpv testing and cell block processing in separate vials containing 5ml of the fixative and centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and 1-2 ml of polymer solution was added. The cell blocks studied were lesser than the liquid based cytology cases, using equal amount of cell block fixative (10% formalin and 95% alcohol) and to compare it with conventional pap smears. In the present study p16 markers were done on cell block
preparation. DNA was extracted from 50 LBC samples using the manual Phenol IsoChloroform method.

Results

conventional pap smear (CPS) vs cell block (CB) is 75 cases vs 50 NILM vs Chronic cervicitis (47/36), LSIL (3/1), HSIL (2/1), SCC (3/3), infections (7/7), Koilocytic atypia (1/1), AGUS (1/1), Unsatisfactory on CPS vs No deposit on CB (5+6/25).

Histopathological correlation was available for 25 cases. Out of which 22 being chronic cervicitis and 3 neoplastic which correlated with cell block diagnosis.

The results for HPV DNA testing done for 50 cases showed 5 cases positive for HPV. 4 cases were low risk HPV while one case was high risk HPV DNA 16.

Conclusion

MLBC is an useful cost-effective method for early detection of cervical cancer in resource poor settings.

References


3 Lynette Denny, Michael Quinn, R. Sankaranarayanan Screening for cervical cancer in developing countries. Vaccine 24S3 S3/71–S3/77, 2006


PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS AMONG 2000 WOMEN IN RURAL GHANA - THE ACCESSING STUDY


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Background / Objectives

To determine the prevalence of 18 sexually transmitted infections (STI) among 18-65 year old women living in the rural North Tongu District in Ghana.

Methods

This population-based study included 2000 women who were representatively selected by geographical distribution and invited to self-collected vaginal samples (Evalyn brush, Rovers). Extracted DNA was tested for 18 STIs by multiplex PCR followed by Luminex bead-based hybridization (STIP Assay, Schmitt et al., 2014, J Infection 69:123).

Results

1937/2000 samples collected during the ACCESSING study had sufficient DNA quality and were eligible for STI analyses. The median age of the study population was 30 years. The most prevalent infectious agents were Gardnerella vaginalis (53.7%), Atopobium vaginae (49.1%) and Mycoplasma hominis (33.7%), all known to be associated with bacterial vaginosis (BV). Using a scoring system (according to Schmitt et al. 2014), 24.4% (472/1937) of the women showed a strong or very strong indication for BV. Ureaplasma parvum and Ureaplasma urealyticum were detected in 50.1% (971/1937) and 30.5% (591/1937) of the samples, respectively. Chlamydia trachomatis, causing pelvic inflammatory disease, was detected in 4.9% (94/1937; 95% CI: 4.0% to 5.9%) of the women and Neisseria gonorrhoeae in 2.5% (48/1937). Trichomonas vaginalis showed a prevalence of 4.1% (79/1937) and Treponema pallidum was detected in one sample only.

Conclusion

Data for BV is very rare in the literature and therefore the prevalence of the infectious agents causing BV along with its scoring system provide a first insight into the
estimated prevalence in Ghana. Prevalence reported for WHO African Region for *Neisseria gonorrhoeae* with 2.3% is similar to what we found in our study population. On the contrary WHO reports a prevalence of 2.6% for *Chlamydia trachomatis*, i.e. 2.3% lower than our data. This difference is statistically significant (p-value < 0.0001). This could possibly be due to the relatively young age group with a median age of 30 years in our study. *Treponema pallidum* with only one case is below the WHO reported prevalence of 3.5%. This differences could be due to the acutely infected vaginal sample used for our analysis, compared to anamnestic serum samples used for the detection of *Treponema pallidum* in many studies.

The rare estimates of STI prevalence in developing countries published in the literature represent a contrast to the high burden of disease associated with STIs. Therefore, this data is of great importance. At the same time it highlights the urgent need for further research in this field, due to varying prevalence rates seen, to guide future public health policy.
The Study of Folate Receptor-Mediated Staining Solution (FRD™) Used for Detecting High Grade Cervical Lesions and Invasive Cancer

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Background / Objectives

To evaluate the sensitivity and specificity of Folate Receptor-Mediated Staining Solution (FRD™) used in detecting high grade cervical lesions and invasive cancer.

Methods

The FRD™ is designed for rapid visualization of CIN2+. Results are determined immediately (within 30 sec) after staining of the entire cervical epithelia. Patients who visited the outpatient clinic were recruited for this study. HR-HPV and cytology tests were performed before FRD™ testing. During the FRD™ testing both the cervix and cervical canal were stained. Colposcopy and biopsy was performed on the patients with either ≥ASC-US cytology test, positive HPV test, or positive FRD™ test. An ECC was completed on patients if the result was positive for the FRD™ test in the cervical canal, cytology result was AGC, or after a colposcopy the transformation zone of cervix was type II, III.

Results

This study involved 404 women. CIN 2+ was found in 65 patients (16.1%) including 9 patients with cervical invasive cancer. CIN 1 and inflammation accounted for 7.4% (30/404) and 76.5% (309/404), respectively. Cytology results included: NILM: 140 (34.7%), ASC-US: 119 (29.5%), ASC-H: 5 (1.2%), LSIL: 81 (20.0%), HSIL: 59 (14.6%). The HPV positive rate was 93.6% (378/404). Positive FRD™ test was determined in 53.2% women (215/404). The sensitivity of cytology, HPV, and FRD™ in detecting CIN 2+ lesions was 90.8%, 96.9%, and 80.0%, respectively. The specificity was 39.5%, 7.1%, and 51.9%, respectively.

Conclusion

The specificity of the FRD™ is the highest, comparing with cytology and HPV test, and the sensitivity is compatible. The FRD™ is suitable for detecting high grade cervical lesions and invasive cancer. Test results are determined immediately (within 30 sec) after staining of the entire cervical epithelia for detecting abnormal cervical lesions (CIN2+). Also, the FRD™ is easy to operate, since operators do not need professional training or professional equipment. Therefore, the FRD™ testing is an accessible and inexpensive method, especially for less-developed countries, and can be used as alternative cervical cancer detecting method.
COMPARISON OF THREE HPV ASSAYS IN DETECTION OF CERVICAL CANCER

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Background / Objectives

Without HPV vaccination, the cervical cancer incidence rate is still increasing in China. Screening remains the primary way to prevent cervical cancer, and there were so many methods that can be used for HPV detecting. This study was to describe the dominated HPV types in squamous cell cancer (SCC) and adenocarcinoma (ADC) in China, and to compare the clinical performance of three assays detecting HPV E6/E7 DNA, E6/E7 mRNA and E6 oncoprotein in cervical cancer.

Methods

487 women were recruited from 4 central hospitals in China, among whom 448 were diagnosed with SCC and 39 with ADC. Specimens of exfoliated cervical cells from these participants were tested by 3 assays: HPV E6/E7 DNA-based detection (BD onclarity) which could test 6 individual HPV genotypes (HPV16, 18, 31, 45, 51 and 52) and 3 groups of hrHPVs (HPV33/35, 35/39/68 and 56/59/66), HPV E6/E7 mRNA detection of 14 HR HPV types (APTIMA) and HPV16/18 E6 oncoprotein strip test.

Results

SCC mainly occurred in women at 45-49 years old, and ADC was most frequently observed in women at 40-44 and 60-64 years old in China. HPV16, HPV18 and HPV33/58 were dominated in SCC, as to ADC, the dominated genotypes were HPV 16, HPV18 and HPV39/68/35. HPV 16/18 accounted for 85.4% cervical cancer in China. HPV DNA test and HPV mRNA test showed the same sensitivity in detection for cervical cancer (94.9% vs. 94.0%, P>0.05). When focused on HPV 16/18, we noticed that HPV 16/18 DNA detection found more cases than HPV 16/18 E6 oncoprotein testing (85.4% vs. 79.3%, P<0.05). And the positivity rate of HPV 16 DNA was significantly higher than that of HPV 16 oncoprotein (77.6% vs. 69.6%, P<0.05), while the positivity rate of HPV 18 DNA was the same as HPV 18 oncoprotein (11.9% vs. 13.6%, P>0.05).

Conclusion
HPV16 and HPV 18 were dominated in SCC and ADC in China, thus HPV16/18 vaccine would protect about 80% Chinese women from cervical cancer if the vaccination was implemented. HPV DNA and HPV mRNA testing showed almost the same sensitivity in detection of cervical cancer, both of which were better than HPV 16/18 E6 oncoprotein testing.
COMPARISON OF VIA with molecular testing using HPV-DNA and the biomarker p16INK4a/Ki-67 for cervical cancer screening in a high-prevalent cervical cancer setting

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Background / Objectives

Cervical cancer is the most common cause of cancer death among women in Kenya. Visual inspection with acetic acid (VIA) has become the standard screening method. However, diagnostic accuracy of VIA is limited due to observer subjectivity and participation in VIA screening program is very low. We therefore compared VIA with molecular screening methods i.e. HPV testing and the p16INK4a/Ki-67 immunocytochemistry, a biomarker specific for HPV-transformed cells.

Methods

In two rural clinics belonging to the catchment area of Moi Teaching and Referral Hospital women who participated in the VIA program offered by the regional health service were invited to also participate in the molecular screening study. Consenting women had a liquid-based cytology sample taken before conducting VIA according to the manual on visual screening for cervical neoplasia published by IARC, 2003. Liquid-based cytology (LBC) samples were used (i) to prepare a microscopic slide using Thinprep® 2000 processor (Hologic®) and (ii) performing HR-HPV DNA (HC2, Qiagen®) assay on the remaining sample. Slides were sent to Heidelberg, Germany, where p16INK4a /Ki-67 immunostaining using Roche® CINtec PLUS® kits was performed.

Results

576 women have been recruited so far. The analysis is done on a preliminary dataset of 321 women. 158 (49.2) women were 30+ years old, 37 (6.2%) were multipara, 33 (10.7%) of 308 women reported to be HIV-infected. The positivity rate of HPV DNA, VIA and p16INK4a /Ki-67 were 30.8%, 4.98%, and 2.5%. 8.1% of all HPV DNA samples were p16INK4a /Ki-67 positive. All p16INK4a /Ki-67 positive sample were
also HPV DNA positive. Of all VIA positive samples 31.3% were HPVDNA positive and none p16INK4a /Ki-67 positive.

Conclusion

The VIA results correlated poorly with both the HPV DNA status and the biomarker p16INK4a /Ki-67. Colposcopic follow-up of all HPV positive women will be done. The use of p16INK4a /Ki-67 as a triage test for HPV DNA positive women will be studied in future.
VULVAR CANCER: TWO PATHWAYS WITH DIFFERENT LOCALIZATION AND PROGNOSIS

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Background / Objectives

There are two etiologic pathways for vulvar squamous cell carcinoma (SCC). The first occurs in a background of lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia (dVIN). The second is related to high-risk human papillomavirus (HPV) infection with its precursor lesion high grade squamous intraepithelial lesion (HSIL). The aim of this study was to investigate the predilection site and survival of HPV-related compared to non HPV-related vulvar SCCs.

Methods

Data of all consecutive patients with primary vulvar SCC treated at the Department of Gynaecologic Oncology at the Radboud university medical center are prospectively stored in a database: data of patients who have been treated between March 1988 and January 2015 were analyzed. All available histological specimens were tested for HPV with the SPF10/DEIA/LiPA25 system assay and p16INK4a immunohistochemical staining was performed using CINtec® histology kit. Vulvar SCCs were considered HPV-related in case of either >25% p16INK4a expression and HPV positive or >25% p16INK4a expression, and HSIL next to the tumour. The tumour localization, disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) of patients with HPV-related and non HPV-related vulvar SCC were compared.

Results

In total 318 patients were included: 55 (17%) patients had an HPV-related vulvar SCC (Group 1) and 263 (83%) patients had a non HPV-related vulvar SCC (Group 2). The tumours in Group 1 were significantly more often located at the perineum compared to Group 2, 30% and 14%, respectively (p = 0.001). The DSS, DFS and OS were significantly better in the HPV-related than in the non HPV-related vulvar SCC patients.

Conclusion

HPV-related vulvar SCCs are more frequently located at the perineum and have a favourable prognosis compared to non HPV-related vulvar SCCs. Both localization of the tumour and the HPV-related pathway could explain the favourable prognosis. HPV-related vulvar malignancies seem to be a separate entity within vulvar SCC.
The role of the antileukoprotease secretory leukocyte protease inhibitor (SLPI) in squamous cell carcinoma of the vulva in relation to HPV-infection and smoking habit of the patients

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Background / Objectives

It was previously shown that protein and mRNA expression of the antileukoproteinase SLPI was significantly inverse correlated with HPV-infection in HNSCC and led to the suggestion that elevated expression of SLPI protects against HPV-infection in HNSCC. In addition we could show that SLPI expression was upregulated in HNSCC patients reporting a smoking habit. Here we investigate whether this inverse correlation between HPV-infection smoking habit and SLPI expression could also be found in other HPV-driven cancers, namely vulvar squamous cell carcinoma (VSCC).

Methods

FFPE samples of 116 VSCC were analyzed by PCR and RT-qPCR for HPV-DNA-, and SLPI mRNA-expression and data were correlated. Data correlating HPV- and SLPI-expression with smoking habit are so far preliminary since at present only 68 patients files are complete. The same holds true for Kaplan-Meyer-analysis correlating HPV-status and SLPI-expression with overall (OS) and progression free survival (PFS).

Results

Of the analyzed 116 VSCC 10 (8.6%) are HPV-DNA positive (Genotyping by Sanger sequencing is ongoing, followed by HPV-RNA-analysis). Of the 68 patients with complete files 24 (35.3%) reported a smoking habit and of these patients 3 (12.5%) were HPV-positive. So far 3 further HPV-positive patients were identified as non-smokers and for the remaining 4 at present no data are available. SLPI-expression however, was independent of the smoking habit, 4.0-fold lower in HPV-positive than HPV-negative patients. Smoking on the other hand resulted, independent of the HPV-status of the patients, in 5.6-fold higher SLPI expression. HPV-positivity and low SLPI-expression are associated with better PSF (analysis of OS data is ongoing).

Conclusion

The data presented here indicate that SLPI plays a pivotal role in HPV-infection not only in HNSCC but also in VSCC and possibly also in other HPV-driven cancers.
This however, needs to be analyzed in future studies. Furthermore these data lead to the hypothesis that the smoking induced SLPI-increase is systemic rather than local, as assumed based on the HNSCC data.
FC 03-03
DNA COPY NUMBER ABERRATIONS ASSOCIATED WITH HPV-DEPENDENT AND -INDEPENDENT VULVAR CARCINOGENESIS

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Background / Objectives

Vulvar squamous cell carcinoma (VSCC) can develop through HPV-dependent (25%) and HPV-independent pathways, indicating a heterogeneous disease. High-grade vulvar intraepithelial neoplasia (VIN) is the precancerous state of VSCC but only a minority of VINs progress to cancer. Current clinical and histological classifications are insufficient to predict the cancer risk. Consequently, affected women are treated similarly with mutilating interventions. Hence there is a clinical need for objective biomarkers reflecting the cancer risk. Here we analysed copy number alterations (CNA) to assess the significance of molecular heterogeneity of vulvar lesions in relation to HPV status and cancer risk.

Methods

25 VSCC and 42 VIN, including VIN of women with associated VSCC (VIN with VSCC) and VIN of women who did not develop VSCC during > 10 year follow-up (VIN without VSCC) were analysed for HPV-status by means of p16\(^{\text{INK4a}}\) immunohistochemistry and HPV testing. CNA were determined by whole-genome next-generation shallow sequencing and CGHcall, CGHregion and CGHtest analysis.

Results

HPV-positive VSCC (n=11) and HPV-negative VSCC (n=14) showed a partially overlapping pattern of recurrent CNA, including frequent gains of 3q and 8q. HPV-negative VIN (n=11) had significantly less CNA (\(P = 0.010\)), mainly consisting of 8q gains and 8p losses. Amplification of 11q13/cyclinD1 was exclusively found in 46% of HPV-negative lesions. In HPV-positive lesions no difference in CNA frequency was found between VIN with VSCC (n=17) and VSCC (\(P = 0.48\)), though CNA were less frequent in VIN without VSCC (n=14; \(P = 0.058\)). Interestingly, almost all (88%) HPV-positive VIN with VSCC had chromosome 1 gain, whereas this alteration was infrequent (21%) in VIN without VSCC.
Conclusion

HPV-dependent and independent vulvar carcinogenesis is characterized by frequent alterations of chromosome 3 and 8, as well as distinct CNA such 11q13 amplification in HPV-independent lesions. The extent of CNA in HPV-positive VIN was found to reflect the cancer progression risk. In particular, gain of chromosome 1 was strongly associated with cancer progression.
Does HPV Genotype Affects the Grade and the Risk of Recurrence of Vaginal Intraepithelial Neoplasia?


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Background / Objectives

Vaginal intraepithelial neoplasia (VAIN) is a rare pre-malignant lesion of the female genital tract. Like cervical squamous intraepithelial lesions, Human Papillomavirus (HPV) infection represents the main risk factor and three grades of VAIN can be identified, according to the depth of epithelial involvement. However, there is still controversial data about HPV detection rate in VAIN and prognostic factors of recurrence and progression to malignancy. The aim of this study was to investigate any correlation between HPV genotype and grade of VAIN and between pre-treatment HPV genotype and risk of recurrence and/or progression.

Methods

Women attending the European Institute of Oncology, Milan, and the IRCCS Fondazione Policlinico San Matteo, Pavia, from January 2000 to December 2016, were enrolled in a multicentre retrospective study. Clinical and histological characteristics of all patients were recorded. Only patients in which HPV testing was performed at the moment of diagnosis were selected. The presence of HPV DNA was evaluated with the Cobas HPV assay in Milan and with the INNO-LiPA genotyping system in Pavia. For our analysis, results of both HPV tests were classified as HPV 16-18, other pooled High Risk (HR)-HPV and negative HR-HPV. The χ2-test was used to evaluate associations and a P-value <0.05 was considered statistically significant.

Results

Among 266 patients enrolled, only 167 diagnosed with VAIN1 (59), VAIN2 (42) and VAIN3 (66) were suitable for this analysis. The median follow-up time was of 23.6 months (95% CI: 18.8 – 30.1). HPV 16-18 was detected in 12 (20.3%) VAIN1, 13 (30.9%) VAIN2 and 37 (56.1%) VAIN3, whereas other HR-HPV in 36 (61.0%) VAIN1, 24 (57.1%) VAIN2 and 21 (31.8%) VAIN3 (p<0.001). Overall, 48 (28.7%) recurrences/progressions occurred, with a median time of progression free survival of 93.4 months. No statistical difference was found in number of relapses between pre-treatment HPV 16-18 (22) and other HR-HPV (21) (p=0.26).

Conclusion
HPV 16-18 represent the main genotypes associated with the development of high-grade VAIN, whereas other HR-HPV are more frequently related to low-grade VAIN. Nevertheless, pre-treatment HPV genotype does not affect the risk of recurrence and/or progression of vaginal dysplasia.
DISTRIBUTION OF HIGH-RISK HPV TYPES IN WOMEN WITH INVASIVE CERVICAL CARCINOMA IN KAZAKHSTAN

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Background / Objectives

In Kazakhstan, cervical carcinoma (CC) is the most common cancer in women aged 15 to 44 years, ranking second as the leading cause of female cancers. According to the National Cancer Registry, the incidence rate of CC in Kazakhstani women is very high. In 2012, the estimated crude incidence rate of CC was 22.8 per 100,000 women with a mortality rate of 9.8 per 100,000 women. Unfortunately, data regarding the prevalence and distribution of high-risk HPV (hrHPV) types in CC in Kazakhstan are scarce. Hence, the aim of our study was to evaluate the distribution of hrHPV types in invasive CC samples obtained from Kazakhstani women.

Methods

A total of 99 archival formalin-fixed paraffin-embedded (FFPE) tissue samples, obtained from the same number of Kazakhstani women with histologically confirmed invasive CC, were included in the study. Total DNA was extracted from three 10 μm tissue sections of each FFPE block using a DNA Mini Kit (Qiagen, Hilden, Germany), following our in-house protocol for DNA extraction from FFPE tissues. Detection of hrHPV types was performed using a RealTime High Risk HPV Test (Abbott, Wiesbaden, Germany), which enables concurrent separate genotyping of HPV16 and HPV18 and pooled detection of 12 other hrHPV types: HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Up to 200 ng of each DNA isolate was used per PCR reaction to allow efficient PCR amplification.

Results

Out of 99 samples tested, two (2.0%) were excluded from the analysis due to invalid results for amplification of beta-globin. In total, 77/97 (79.4%) samples tested positive for the presence of hrHPV types. HPV16, HPV18, and other hrHPV types were present in 69/77 (89.6%), 3/77 (3.9%), and 6/77 (7.8%) samples, respectively. An infection with a single hrHPV type was detected in the majority of samples (98.7%).

Conclusion

To the best of our knowledge, this is the first study to evaluate the distribution of hrHPV types among Kazakhstani women with invasive CC. The prevalence of hrHPV types in FFPE CC samples was slightly lower compared to previous studies, most likely due to
the fixation process and/or storage conditions. However, approximately 80% of samples tested positive for the presence of hrHPV types, of which HPV16 was detected in almost 90% of cases. Nevertheless, further studies evaluating the distribution of hrHPV types in fresh tissue samples are needed to confirm our observations. Our data suggest that the implementation of HPV vaccination could have an enormous impact on the incidence rate of CC in Kazakhstan.
PHYSICAL ACTIVITY, OBESITY AND CERVICAL CANCER IN GERMANY

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Background / Objectives

The incidence of cervical cancer in Germany remains high in relation to other countries in Western Europe. Germany currently does not have an organized cervical cancer screening (CCS) program and screening efforts with the Pap smear remain opportunistic. Recent studies have shown levels of physical activity and sport to be associated with cancer prevention, although data on the association with cervical cancer remain limited. The TeQaZ study is a case-control study investigating participation in CCS. Associations between cervical cancer and other risk factors, such as physical activity and obesity, were investigated.

Methods

Incident cases of cervical cancer, diagnosed between 2012 and 2016 in different regions in Germany, were recruited. Cases were matched with three population-based controls, recruited via population registries, based on age and region of residence. Gynecologists were asked to report frequency of CCS participation during the past ten years. Socio-demographic and other risk factors were assessed in cases and controls via telephone interviews. Physical activity was defined as engaging in any form of movement such as walking stairs and doing housework. Additionally, sport activity was documented and Body Mass Index (BMI) calculated. Conditional logistic regression analyses were performed.

Results

A total of 218 cases and 654 controls were included in the analysis. 94.5% of cases engaged in any kind of physical activity at least 30 minutes a day compared to 92.2% of controls. 21.2% of cases and 21.1% of controls participated in sport at least three times a week. With regards to obesity, 20.6% of cases versus 12.2% of controls had a BMI over 30. When adjusting for additional factors, participating in sport at least three times a week and engaging in any physical activity at least 30 minutes a day did not show any preventive effect. However, BMI >=30 and participation in CCS less frequently than every three years were strong risk factors for cervical cancer. However, only 52.1% of women with a BMI over 30 participated in CCS at least every three years, compared to 71.6% of women who were not overweight (p<0.05). Results of the conditional logistic regression will be presented.

Conclusion
Initial findings suggest that physical activity and sport are not associated with developing cervical cancer. An association between BMI and cervical cancer was found, although this may be due to decreased participation in CCS among overweight women.
FC 03-07
TEN YEARS STUDY OF INVASIVE CERVICAL CANCER: MICROINVASIVES CASES INCREASE IN CO-TESTING PERIOD

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Background / Objectives
To study the impact of Human Papillomavirus (HPV) screening plus cytology (co-testing) in the detection of invasive cervical carcinomas in a low incidence area in the north of Spain, adapting the Spanish Society of Gynaecology and Obstetrics (SEGO) scientific protocols.

Methods
Area served by Hospital of Barbastro: A target population of 27,490 women between 25 to 65 years. Period of time: From January 1st 2006 to December 31st 2010 (G1) screening was performed with cytology and from January 1st 2011 to December 31st 2015 (G2) the screening was with cytology and HPV test. The detection of HPV has been through Hybrid Capture (HC2) until the end of 2011 and PCR hrHPV-DNA with Cobas 4800® later. The follow up was until April 30th 2017. The patients came from Primary Care (PC) screening and from gynaecology service screened according to SEGO 2006 protocol (only cytology) for G1 and to 2010 protocol (co-testing) for G2. Demographic, pathological and clinical characteristics were studied.

Results
A total of 30 invasive cases were detected. In G1, 22,888 cytologies (69.6% in PC) and 1,877 HPV tests (43.0% in PC) were performed. The mean age was 45.9 years. 12 (40%) invasive cases were detected in this group. 4 patients (33.3%) died with a survival average of 37.9 months. In G2, 22,740 cytologies were performed (83.5% coming from PC) and 17,209 HPV tests (80.5% in PC). The mean age was 48.6 years. 18 invasive cases were detected, 4 patients died (22.2%) and the survival average was 7.6 months. In G1 the histological type was squamous carcinoma in all
except 1 neuroendocrine carcinoma. 3 (25%) cases were A1 stage. In G2, 7 adenocarcinomas and 11 squamous carcinomas were diagnosed. 6 (33.3%) cases were A1 stage.

Conclusion

In G2 period more microinvasive cases and adenocarcinomas were detected. This increase is related mainly to screening based in Primary Care and co-testing. Mortality has not changed despite the detection of a higher number of cases due to early stages at the moment of diagnosis. 5 years are not enough to know the impact of early detection in mortality.
What is the impact of the HPV vaccination program on the natural history of high grade squamous intraepithelial cervical lesions in New Zealand?

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Background / Objectives

A free quadravalent HPV vaccination program for young women commenced in 2008, publicly funded vaccinations are registered in the NZ vaccination registry. Current recommendations for cervical screening include cervical cytology tests every 3 years for all women over the age of 20. Subsequently we have seen a modest reduction of high grade abnormalities in young vaccine eligible women. However as vaccination protects against only 2 oncogenic HPV types, has this resulted in a change in type distribution of HPV among women with high grade abnormalities? If this is so what if any are the clinical implications of this change?

Methods

To explore this question we will present data from 2 studies. The first is a matching of data from the NZ vaccination register and the National Cervical screening register. The second is a large multicenter study of over 600 women under the age of 25 undergoing observational management in young women with CIN2 ref 1.

Results

In New Zealand approximately 60% of women aged between 20 and 25 have received at least 2 doses of Gardasil ©. Annually in NZ approximately 53,000 cytology samples are taken in women under the age of 25, 16% of which are reported as abnormal. Approximately 1000 high grade biopsies were reported in women under the age of 25 annually. By matching data from these registries we are able to determine that women who were vaccinated had a lower rate of high grade histological abnormalities and there was an overall trend to a lowering rate of high grade histology.

In the population of young women with high grade histological abnormalities women taking part in our observational study the proportion of women with HPV 16/18 related lesions has fallen from 40-12%. This fall has been most marked in non vaccinated women.
**Conclusion**

While HPV vaccination has resulted in only a modest decrease in high grade cytological abnormalities in young women there is evidence to suggest there has been a rapid decrease in the proportion of these abnormalities caused by HPV 16 and 18. There is evidence to suggest that non 16/18 lesions have a more benign course. We suggest therefore this has implications for screening and the treatment of high grade abnormalities in young women.

**References**


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Background / Objectives

Infants born preterm are at increased risk of morbidity and mortality. Conization for the treatment of cervical dysplasia has been associated with an increased risk of preterm delivery. However, valid comparable data for Germany are still missing. Our study aimed to investigate the association between conization and perinatal outcomes in subsequent pregnancies, using data from a German population database.

Methods

A retrospective cohort study was performed on data from the German nationwide performance measurement program in healthcare quality. The survey routinely collects parameters of women who give birth in a German hospital. Approximately 98.5% of all births in Germany are covered within the data collection, comprising a total of 4,002,503 births between 2009 and 2014. Women with history of conization prior to pregnancy were compared to a control group of women without. To control for multiple pregnancies the cohort was limited to singleton deliveries and to avoid double-counting, only primipara were included. Main outcome measures are gestational age at birth, birth weight, neonatal morbidity and perinatal mortality. Data were analyzed using univariate and multivariate statistical methods.

Results

A total of 1,573,200 cases were eligible for inclusion. There were 14,337 women with history of conization and 1,328,057 women without. Women with history of conization were more likely to be single, (self-) employed, older, had a lower body mass index and delivered infants with lower birth weight [mean (SD), 3.240g (± 603g) vs. 3.307g (±545g), p < 0.0001]. The preterm birth rate was significantly higher in the conization cohort compared to the non-exposed cohort (12.2% vs. 7.5%; Chi²<0.0001).
Conization was a significant risk factor for preterm birth (odds ratio, OR 1.7; 95% CI: 1.65-1.83). There was no significant difference in stillbirth and death after 7 days of birth between both groups (OR 0.9, 95% CI: 0.66-1.25; OR 1.6, 95% CI: 0.92-2.65).

**Conclusion**

Pregnancies complicated by conization are at a greater risk of preterm delivery. There was no increase in perinatal mortality. Further research of the data set will investigate whether preterm delivery after conization affects the perinatal morbidity.
Correlation of isotope count with sentinel node positivity in vulvar cancer


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Background / Objectives

Sentinel node biopsy (SNB) has become standard of care in early stage vulvar cancer. As the correlation of isotope count with the presence of metastases remains unclear, often several active nodes are excised per groin. This can result in increased morbidity in node-negative disease despite of SNB. In the current analysis we assess, whether resection of the hottest node could be sufficient to detect sentinel lymph node (SNL) metastasis.

Methods

All patients with primary vulvar cancer receiving a SNB with radioactive tracer at the University Medical Center Hamburg-Eppendorf between 2008 and 2015 were evaluated. The day before surgery, patients received four peritumoral intradermal deposits at 3, 6, 9 and 12 o’clock with an overall mean dosage of 85±12MBq99mTc–nanocolloid. Planar lymphscintigraphy was performed one hour after injection. Intraoperatively, a handheld gamma counter was used to identify the SNL.

Results

145 patients with SNB were included; thereof 144 underwent bilateral SNB, resulting in 289 analyzed groins. A median of 2 (range 1-7) SNL per groin were removed. From 94/289 (32.5%) groins more than 2 SNL were excised. Median overall SNL isotope count was 1400. In 50 groins, a positive SNL was detected (unilateral in 38 patients, bilateral in 6). The median number of positive SNL per groin was 1 (range 1-4). The SNL with the highest isotope count carried metastases in 36/46 groins (78.3%; in 4 cases the highest count was unknown). In 10/46 (21.7%) positive groins, the SNL with the highest count was not the metastatic SNL (9/10 second highest count). Median count of these 12 SNL was 60% of the highest count with a range from 11.0% to 74.0%.

Conclusion

The highest isotope count does not reliably detect the positive SNL in vulvar cancer. To prevent mostly fatal groin recurrences, surgeons should continue to remove all SNL accumulating relevant radioactive tracer over minimal background activity.
PREVALENT AND INCIDENT CANCERS IN HPV NEGATIVE WOMEN

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Background / Objectives

HPV testing is better than cytology in primary screening because it reduces subsequent cancer incidence. Moreover, the long-term CIN3+ risk is lower after a negative HPV test than after normal cytology, justifying a longer screening interval in older women who are less likely to become infected. The main concern with a screening interval of up to 10 years in HPV negative women aged over 40 is their invasive cancer risk, which is about 1 in 1,000 over 10 years.

Methods

In the ARTISTIC Trial 24,510 women attending for routine cervical screening in 2001-2003 had cytology and HPV testing at entry and 3-yearly until 2009. We have followed the cohort to 2015 through national cancer and CIN3 registration.

Results

Respective numbers of CIN3s and cervical cancers by age at diagnosis were 208 and none aged 20-29, 192 and 7 aged 30-39, and 83 and 15 aged 40-65. Six of the 22 women with cancer were HPV negative by HC2 at entry, but on retesting their stored entry samples by PCR 5 were HR-HPV positive. 2 of these 6 cancers were diagnosed within 5 years of the negative HC2 test and were probably present at entry. At entry one was cytologically abnormal and both had HPV16 detectable by PCR.

Conclusion

The important measure of efficacy with a long screening interval is the cancer risk, not the CIN3 risk. Most cancers caused by subsequent HPV infection will develop towards the end of the interval due to the lag from infection and CIN3 development to malignancy. These are likely to be diagnosed at an early stage even with a 10-year interval. However, cancers present at the time of the negative HPV test would be at high risk of being advanced or metastatic 10 years later. The results of ARTISTIC and other large studies suggest that most of these prevalent cancers would be detected by more sensitive HPV testing and/or cytology co-testing, but the numbers are small even in very large studies. Collaborative analyses focussed on this issue are needed to provide evidence on the effects on early and advanced cancer incidence of enhanced testing procedures with screening intervals of up to 10 years at different ages, and particularly at a woman’s final HPV test. Modern HPV tests
may already be sensitive enough to prevent most of the small but serious hazard of undetected invasive cancer.
REINVESTIGATION OF A PROPORTION OF HPV-NEGATIVE TUMORS IN A SWEDISH COHORT OF CERVICAL CANCER

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Background / Objectives

Most cervical cancer develops as a result of a permanent infection with human papillomavirus virus (HPV). Despite the common perception that HPV is a requirement for the development of cervical cancer, a smaller proportion of HPV negative cervical cancer is often found in larger studies.

The aim of this study was to reinvestigate a proportion of HPV-negative tumors in a Swedish cohort (n=209) of patients diagnosed with cervical cancer, previously analyzed for detection of HPV where 14.4 % of the tumors were found to be negative.

Methods

Cervical cancer tumors with a HPV negative or invalid result from genotyping with AnyplexTM II HPV28 (Seegene) were included (n=37). This real time PCR method targets 28 genotypes using the viral L1 gene together with the human gene HBB. Second approach included an in house real time PCR protocol instead targeting the viral oncogenes E6 or E7 for 12 high-risk and two low-risk genotypes.

Samples with HPV negative results with both real-time PCR methods were assessed by pathologist and tumors with lacking amount and quality were excluded. Remaining HPV-negative samples were investigated with immunohistochemistry (p16, Vim, ER, PR, CD10, CEA, CK5, P63 and MUC2) to exclude the inclusion of tumors of non-cervical origin.

Results

The initial results showed a proportion of 14.4 % negativity. With repetitive analysis (Anyplex) and second approach with alternative genotyping method the HPV-negativity was 9.6 %. After assessment of tumor material together with immunohistochemistry, five samples were excluded due to lack of tumor material or suspicion of other than cervical origin. This resulted in a HPV-negative proportion of 7.2 % in this Swedish cohort of cervical cancer. HPV-negativity was significantly
(Pearson chi-square test; $p < 0.0001$) associated with adenocarcinoma (AC) histology and worse cancer-specific survival rate at 5 years (log-rank test; $p = 0.010$).

**Conclusion**

Reinvestigation of HPV-negative samples led to a drop of the total proportion of HPV negative tumors from 14.4 % to 7 %. HPV negativity in this group was associated with poor prognosis.
Background / Objectives

HPV-negativity has been reported to be associated with worse prognosis for some HPV-associated cancers. Whether detectability of HPV is related to prognosis of invasive cervical cancer is more controversial and would need very large studies to be clearly answered.

Methods

We identified all cervical cancers diagnosed in Sweden during a 10 year period (2002-2011; 4254 confirmed cases), requested the archival blocks and subjected them to HPV genotyping using general primers targeting the L1 region, followed by typing with Luminex for 14 high risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and non-high risk types including 6, 11, 26, 30, 40, 42, 43, 53, 54, 61, 67, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, and 91). Blocks from 2848 cases were retrieved and analyzed, and were prospectively followed up from date of cancer diagnosis to 31 December, 2015, migration from Sweden, or death, whichever occurred first. Five year relative survival ratios were calculated and excess hazard ratios (EHRs) with 95% confidence intervals (CIs) were estimated by a Poisson model, adjusted for age at cancer diagnosis, FIGO (International Federation of Gynecology and Obstetrics) stage and education.

Results

The HPV L1 region was detected among 2368 (83.1%) of all cases. For HPV L1-negative women, the 5-year relative survival ratio was 0.54 (95% CI, 0.49-0.59) and for women with HPV L1- positive tumours 0.74 (95% CI, 0.72-0.76), yielding a crude EHR of 0.45 (95% CI, 0.38-0.53) and adjusted EHR of 0.53 (95% CI, 0.45-0.62). The 5-year age-specific adjusted EHRs for women with HPV L1-positive tumor were 0.53 (95% CI, 0.32-0.88) at age 30-44, 0.69 (95% CI, 0.48-0.99) at age 45-59, 0.59 (95% CI, 0.44-0.79) at age 60-74 and 0.40 (95% CI, 0.30-0.52) at age 74 and above respectively compared to women with negative tumours. Compared to negative
tumours in each stage, the adjusted EHRs of HPV L1-positive tumours were IA: 0.67 (95% CI, 0.08-5.69), IB: 0.61 (95% CI, 0.41-0.91), II: 0.47 (95% CI, 0.34-0.65) and III+: 0.53 (95% CI, 0.43-0.66). For squamous cell carcinoma the adjusted EHR of HPV L1 positive tumours was 0.57 (95% CI, 0.46-0.70), while for adenocarcinoma it was 0.52 (95% CI, 0.36-0.74).

Conclusion

Women with tumors negative for HPV L1 have much worse prognosis than women with HPV L1-positive tumours, irrespective of age, clinical stage and histological tumour type.
THE RELATION BETWEEN HRHPV-NEGATIVE HIGH-GRADE CYTOLOGICAL LESIONS AND HISTOLOGY: A SYSTEMATIC REVIEW.

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Background / Objectives

There is a consensus that a persisting infection with human papillomavirus (HPV) is the main causative agent for the development of a cervical carcinoma. Testing for HPV therefore plays an important role in cervical cancer screening. Suggestions have been made to introduce the HPV test as the primary screening method, replacing cytology as the current screening technique. However, there is discussion on the value of HPV-negative high-grade cytological lesions.

It is of vital importance to establish whether using an HPV test as the primary screening method for cervical cancer will result in missing high-grade cytological lesions, which are the precursors of cervical carcinomas. This systematic review gives an overview of the percentage of women with high-grade lesions on cytology (ASC-H and HSIL) and a negative HPV test, who present with moderate or severe dysplasia or with a malignancy on histology.

Methods

A comprehensive literature search was carried out, including MEDLINE (PubMed, 1 January 2001 until 25 November 2016) and the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library). 925 titles and abstracts were screened. 40 eligible studies included women presenting with a cervical cytology result of ASC-H or HSIL and an HPV-negative test result, who were subsequently subjected to reference standard verification with colposcopy and colposcopy directed biopsies for histologic verification. Two review authors independently extracted data from the selected articles and assessed the quality of the studies. Disagreements were resolved by consensus.

Results

The percentage of females who develop moderate to severe dysplasia or a malignancy (CIN2+) from HPV-negative ASC-H is 10.7%, 95%CI [5.3; 16.0]. The percentage CIN2+, originated from HPV-negative HSILs, is 35.9%, 95%CI [28.4; 43.4].

Conclusion

Histologically confirmed CIN2+ lesions out of an HPV-negative high-grade cytological population are an existing, yet insufficiently studied entity. Consequently, it might result in missing high-grade precursor lesions when HPV testing would be used as a unique
primary screening test for cervical cancer. Additional research is needed to establish the prevalence and the importance of HPV-negative high-grade cytological lesions in Belgium in order to determine the most appropriate screening method.
FC 04-05
HPV-NEGATIVE CARCINOMA OF THE UTERINE CERVIX: A DISTINCT TYPE OF CERVICAL CANCER?

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Background / Objectives

Small proportion of cervical cancers (CC) are negative for human papillomavirus (HPV), even using highly sensitive HPV tests. It has been suggested that HPV-negative CC may represent a biologically distinct subset of tumours carrying a poorer prognosis. However, the significance of HPV-negativity in CC remains unclear. We aimed to provide insight into the differential clinical, pathological, and prognostic characteristics of the unusual HPV-negative CCs.

Methods

A cohort of 215 women with CC diagnosed in the Hospital Clinic (Barcelona) from 1999 to 2014 underwent HPV testing using: 1)a highly sensitive polymerase chain reaction (PCR): SPF10PCR/DEIA/LiPA25 system for HPV-DNA detection and genotyping and 2)p16INK4a immunostaining. Clinical, histological and immunological characteristics of the women included were recorded.

Results

Twenty one out of 215 tumors (9.8%) were negative for HPV-DNA detection. Nine of them (9/21;42.9%) showed also a negative p16INK4a immunostaining result. These double negative tumors were considered as confirmed HPV-negative CC. Within the confirmed HPV-negative CC, 5 were squamouscarcinoma, 2 were adenocarcinoma and 2 were neuroendocrine. Women with confirmed HPV-negative CC were diagnosed at advanced FIGO stage and showed worse disease free survival [47.5 months (95%CI:8.7-86.22 months) vs. 129.6 months (95%CI:116.22-143.01 months); p=0.009] and overall survival [72.1 months (95%CI:25.44-118.80 months) vs. 151.4 months (95%CI:139.70-163.05 months); p=0.056] than women with HPV-positive tumours.

Conclusion

DNA-HPV negative result is an uncommon finding in women with CC, and almost half of these cases show a positive p16INK4a immunostaining. Confirmed HPV-
negative CC seems to be associated with advance FIGO stages and worse prognosis.
IMPLEMENTATION VALIDATION OF THE PAPILLOCHECK® (GREINER BIO-ONE) KIT FOR GENOTYPING HUMAN PAPILLOMA VIRUSES (HPV) IN PRESERVNCYT LIQUID MEDIUM

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Background / Objectives

Persistent infection of the uterine cervix by the same type of high-risk human papillomavirus(es) (HR-HPV) is related with cervical cancer. It becomes increasingly important to know which HR-HPV type(s) is/are present in the cervical smear. The objective of this study is to evaluate the analytical performance of the PapilloCheck kit for the detection and genotyping of 18 high risk and 6 low risk HPVs.

Methods

DNA from patient samples was extracted using the MagNA Pure platform (Roche DNA I High Performance protocol): 1 ml sample was first concentrated by centrifugation (20 min, 20,000 g). 800 µl supernatant was removed and the remaining 200 µl was used for extraction.

Due to the low concentration of cell material of the Quality Control for Molecular Diagnostics (QCMD) panel, the whole sample (5 ml) was concentrated (20 min, 4000g). DNA was eluted in 110 µl elutionbuffer.

DNA of the WHO Proficiency Panel 2011 (PP) was already extracted. 5 µl DNA was used for the PCR.

The assay was checked for analytical sensitivity, specificity, accuracy and precision following the Belgian guidelines.

Results

Analytical sensitivity: a negative PreservCyt specimen was spiked with the WHO-HPV16 DNA standard to determine the limit of detection (LOD with a 95% hit rate). The lowest concentration was 13.333 international units (IU)/ml, correlating with 120 copies/PCR.

Looking at the results of the WHO PP we can assert that the PapilloCheck detects 50 genome equivalent (GE)/PCR of HPV6, HPV11, HPV33, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV68. For HPV18, HPV31 and HPV35 the sensitivity was 500 GE (IU)/PCR. HPV16 was detected at 5 IU/PCR. The PP was designed for genotyping needs in HPV vaccinology. The test is not proficient for HPV18 (50 IU/PCR) but according to the manual the LOD for HPV18 is 300 IU/PCR and is in accordance with the clinical needs.
**Specificity:** the specificity was sufficiently documented by the manufacturer, and was not tested again.

**Accuracy:** 52 specimens were tested.

WHO PP2011: 5 out of 43 were false negative (lower than LOD). QCMD 2011 panel: 8 out of 9 were typed correctly. HPV45 in cc10b cells was missed. This cell line does not contain the full target used in the assay. HPV45 was 4/4 times detected in the WHO PP.

**Precision:** One sample positive for HPV 31 and HPV51, a second sample with a multiple infection of 3 types: HPV81, HPV33 and HPV73 were extracted in triplicate on 3 different days. All types were detected correctly. This met our validation criteria.

**Conclusion**

The PapilloCheck method met all our validation criteria and was implemented in our routine diagnostic laboratory.

**References**

Raymaekers et al., Acta Clinica Belgica, 2011
A COMPARISON OF THE PERFORMANCE OF PAPTYPE USING CYTOFLEX AND ATTUNE FLOW CYTOMETER PLATFORMS ON CERVICAL SCREENING SAMPLES COLLECTED FROM PRESERVICYT

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Background / Objectives

To compare the performance of PapType, a bead-based full genotyping DNA assay for 14 high-risk (hr) and 2 low-risk (lr) HPV types, using two flow cytometer platforms - CytoFLEX and Attune.

Methods

Residual material from the PreservCyt samples of 6000 women attending for routine cervical screening sent to the cytology laboratory at St. Mary's Hospital, London were tested using PapType HPV test kit (Genera Biosystems, Scoresby, Victoria, Australia). The samples were run on CytoFlex (Beckman Coulter) and Attune Acoustic Focusing (Thermo Fisher Scientific) flow cytometer platforms following the manufacturer's instructions. These samples were collected for the Predictors 3 Study1. The discordance between the platforms was analysed by hrHPV type. Comparisons were also made of sensitivity and specificity for CIN2+.

Results

98.2% of cases gave an adequate result on Attune versus 99.1% on Cytoflex. Overall 17% of Attune cases were positive for at least 1 hrHPV type, and 21.1% of Cytoflex cases were positive for 1 or more hrHPV type. Overall agreement between Attune and Cytoflex was good for detecting hrHPV types (kappa 72.4, 95% CI (70.1, 74.6)). Cytoflex called more cases positive than Attune (significant difference for HPV types 16, 18, 45, 52, 58, 66 and 68). Attune had statistically significantly more positives for HPV 59. There was a wide range of agreement for individual HPV types (kappa 41.1 for HPV 18 to kappa 92.1 for HPV 33). Overall for CIN2+ cases only 1 case was discordant (positive for Cytoflex, negative for Attune). CIN2+ sensitivity was higher for CytoFlex (97.5 vs 95.0). Specificity was higher for Attune (<CIN2 83.5% vs 79.4%).

Conclusion
These preliminary data suggest that both Attune and CytoFlex are reliable flow cytometry platforms on which to run the PapType test. Further HPV type specific and signal strength data will be presented in the final analyses.

References

THE TRANSITION FROM HC2® TEST TO COBAS® 4800 TEST IN THE HPV PRIMARY SCREENING OF THE FLORENTINE AREA

A. Mongia, C. Sani, G. Pompeo, G. Fantacci, E. Burroni, S. Bisanzi, L. Ventura, F. Carozzi

ISPO (Cancer Prevention and Research Institute) (Italy)

Background / Objectives

In cervical cancer screening, HPV test detects DNA of 12 high risk (hr) HPV types (16/18/31/33/35/39/45/51/52/56/58/59) and, depending on test, HPV with probable (68) or possible (66) risk. Among clinically validated tests, Hybrid Capture®2 (HC2®, QIAGEN®) detects 13 HPV types (hr and 68) by in solution hybridization, while Cobas®4800 (ROCHE®) detects 14 types (also 66) in Real-Time PCR. In the Florentine area, from May 2016, HC2® test has been replaced by Cobas® test.

The objective of the study is to evaluate the impact of the transition from HC2® to Cobas® on the HPV primary screening of the Florentine Area, comparing: HPV positivity, cytology triage abnormalities and histological results to immediate colposcopy.

Methods

We considered samples from women participating to HPV primary screening program of the Florentine area (34-64 years), collected in ThinPrep®(HOLOGIC®) from June 2015 to March 2017. Until May 2016, samples were analysed by HC2® on automatic instrumentation (QIASymphony®/RCS®, QIAGEN®). From June 2016, samples were analysed by Cobas®.

Results

The samples collected in ThinPrep® during the considered period were 37775, of which 17137 (45.4%) tested on HC2® and 20638 (54.6%) on Cobas®. The average age of women is similar (46.5 vs 46.1).

HPV positivity was 9.8% (1677/17137) for HC2® samples and 7.4% (1529/20368) for Cobas® samples (p<0.0001).

480 HC2® positive women (28.6%) had abnormal cytology triage and 17 (1%) inadequate; 418 Cobas® positive women (27.3%) had abnormal cytology triage and 21 (1.37%) inadequate.

Adhesion to colposcopy was 90.3% (449/497) in HC2® group and 77.4% (340/439, of which 14 are waiting for histology) in Cobas® group until now, as several women are waiting to perform colposcopy.
For the 449 women of HC2® group, compared to the 326 women of Cobas® group, at the immediate colposcopy we found: 108 vs 118 CIN2+ (PPV: 24.1% vs 36.2%, p<0.0002), 145 (32.3%) vs 88 (27%) CIN1, 195 (43.4%) vs 118 (36.2%) normal colposcopies/histologies (p<0.05) and 1 (0.2%) vs 2 (0.6%) inadequate histologies.

Conclusion

The use of HC2® as primary screening test, compared to Cobas®, has registered: greater HPV positivity, lower CIN2+ PPV at the immediate colposcopy and higher frequency of normal colposcopies/histologies (all statistically significant differences). These results could be explained by the well known HC2® cross-hybridization with non-hrHPV types, unlike Cobas®, which has a higher analytical specificity. Non-hrHPV types detected by HC2® but not by Cobas® likely increase HPV positivity and abnormal cytologies, but decrease PPV at the immediate colposcopy, since they are mostly associated with no lesions or low grade lesions (CIN1), as resulted by our data.
COMPARISON OF VALIDATED MOLECULAR METHODS FOR HPV PRIMARY SCREENING TEST: HC2® TEST VS. COBAS® 4800 TEST.

G. Pompeo, A. Mongia, G. Fantacci, C. Sani, S. Bisanzi, E. Burroni, F. Carozzi

ISPO (Cancer Prevention and Research Institute) (Italy)

Background / Objectives

In the Cancer Prevention Regional Laboratory of ISPO (Florence, Italy), after the transition from Hybrid Capture® 2 test (HC2®, Qiagen®), that detects 13 HPV types (12 HR-HPV+HPV68), to Cobas® 4800 HPV test (Roche®), that detects 14 HPV types (12 HR-HPV+HPV66 and 68), a decrease in HR-HPV positivity was observed in all the Local Sanitary Areas on which the HR-HPV test was already performed by our laboratory. The aim of this study is to compare the performance of the two methods using the same set of samples.

Methods

620 routine screening samples, HC2® positive, were retested on Cobas®. The samples that resulted negative to Cobas® (discordant) were typed by a Reverse Line Blot (RLB) method (Ampliquality HPV-Type express 3.0®-AB Analitica®), that detects the presence of 40 HR and non HR-HPV types. These results were linked with cytological and histological data.

Results

419 samples (67.6%) was confirmed HR-HPV positive to re-test with Cobas®, while 201 samples (32.4%) resulted HR-HPV negative (discordant). This decrease of the positivity to HR-HPV is also confirmed in the screening samples analyzed after the introduction of Cobas®.

The discordant samples reported the following results of cytology triage: 165(82.1%) resulted with normal cytology and 36(17.9%) with abnormal cytology (31/36 LSIL (86.1%), 3/36 ASC-H (8.3%), 1/36 HSIL (2.8%), 1/36 AGC (2.8%)).

All discordant samples were typed using RLB. 14/201 samples(7%) resulted HR-HPV positive by typing and only one of these (HPV58+) had an abnormal cytology (LSIL), but resulted negative to colposcopic exam. The typing results of the other 187 discordant samples were: 88/187(47%) HPV negative, of which 5 with abnormal cytology triage (2 ASC-H, 1 AGC, 2 LSIL) and none with CIN2+ lesion; 99/187(53%) were non HR-HPV positive, of which 30(30.3%) with abnormal cytology triage (1 ASC-H, 1 HSIL and 28 LSIL) and 2 with CIN3 lesions (HPV26 and HPV54+73+90 respectively).
Furthermore, a considerable number of discordant samples (20/201, 10%) resulted HPV68a positive by RLB (5 with abnormal cytology and none CIN2+).

**Conclusion**

Cobas® is a reliable method and is more specific than HC2® (92.5% of discordant samples are HR-HPV negative, so we would have registered a lower rate of false HR-HPV positive samples). Between Cobas® HPV negative samples, but HC2® HR positive, we found two CIN3 lesions resulted associated with LR-HPV types by PCR RLB. HPV 68 is a target type for Cobas® but it is not clear if 68a and 68b are detected at the same level, because, in our set of samples, several HPV 68a resulted negative by Cobas®.
COMPARISON OF THREE DIFFERENT SYSTEMS TO TEST FOR THE PRESENCE OF A HR-HPV INFECTION IN SYMPTOMATIC AND FOLLOW-UP PATIENTS.

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Background / Objectives

As part of the former screening policy in the Netherlands, an hrHPV test is performed on cytological material in symptomatic patients, in triage after ASCUS/LSIL cytological screening results and post-CIN follow-up. Aim of the present study was to compare 3 HPV assays for the detection of hrHPV in smears from these patient groups.

Methods

A total of 1265 residual PreservCyt cervical cytological samples taken either because of symptoms or for follow-up reasons were rendered anonymous, randomized and tested for hrHPV using three automated HPV assays on their respective platforms: QIAGEN’s digene® HC2 HPV DNA Test® (HC2, signal amplification), Roche Cobas® HPV test (DNA amplification) and Hologic Aptima® HPV Test (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. Additionally, inter-assay agreement on hrHPV positive smears was determined for the 3 assays.

Results

1151 samples had valid results in all of the 3 tests. The majority of patients was between 29 and 53 years old (85.8%, n=988). Of these patients 59.7% (n=688) had normal cytology, in 23.6% (n=272) ASCUS was found, 9.3% (n=108) had LSIL and 7.0% (n=81) had HSIL or invasive cancer. Analysis of the results yielded an hrHPV prevalence with Aptima of 41.1% (n=473), with HC2 of 47.9% (n=551) and with Cobas of 44.4% (n=511). Kappa coefficients of 0.81, 0.83 and 0.77 (HC2 vs Cobas, Cobas vs Aptima and Aptima vs HC2, respectively) indicate substantial agreement between the results generated. With increasing degree of cytological abnormalities the hrHPV prevalence rose from 25.8% in normal cytology to 95.7% in HSIL. The kappa values also improved. With regard to inter-assay agreement on hrHPV positive samples, 71.6% (n=426) tested positive in all 3 assays, whereas the percentage of cases that tested positive with a single method was 13.6% (n=81) and the percentage that tested positive with two methods was 14.7% (n=88). The level of
hrHPV positivity in these groups is presently under investigation. Interestingly preliminary results showed that with respect to age there were 2 peaks in hrHPV prevalence (respectively at 20-29 years and in the over 60’s): Final analysis of the results will include a further division based on specific clinical indications.

Conclusion

As expected, an high hrHPV prevalence was found in this symptomatic patient group, with rising hrHPV positivity with increasing severity of cytology. Based on kappa-values the 3 assays showed substantial agreement.
The concordance of HPV DNA and HPV oncogenes mRNA in adenocarcinoma and squamous carcinoma of cervix

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Background / Objectives

The causative role of high-risk human papillomavirus (HR-HPV) in cervical cancer development is well recognized, but HPV infection was less common in cervical adenocarcinoma (CADC) than squamous cell carcinoma (SCC) and CADC is diverse pathologically and in HPV status. Nevertheless, most studies to date have focused primarily on viral DNA rather than the viral transcription. The aim of this study was to investigate the presence of HR-HPV in cervical cancer tissues at HPV DNA level and HPV oncogenes mRNA level by polymerase chain reaction (PCR) and in situ hybridization (ISH) respectively.

Methods

We studied DNA and mRNA levels of HPV in paraffin-embedded samples from patients with CADC and SCC. 60 cases of CADC and 14 cases of SCC were included. Cases were tested for HPV using whole-tissue sections (WTS) and laser-capture microdissection (LCM). All cases were HPV-tested by L1 based broad-spectrum SPF10-DEIA-LiPA25 PCR. HR-HPV mRNA was assayed by novel RNAscope ISH. Type-specific oligonucleotide probes were used for the RNA detection of HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82.

Results

HPV DNA was detected in all 14 (100.0%) SCC and in 36 out of 60 (60.0%) CADC cases by WTS-PCR. Overall, the HR-HPV mRNAs was detected in 12 out of 14 (85.7%) SCC and 20 out of 60 (33.3%) CADC by RNAscope ISH. 20 out of 36 (55.6%) WTS-PCR HPV DNA+ CADC cases detected HR-HPV mRNAs. The remaining 24 (100%) cases of WTS-PCR HPV DNA- CADC were also HPV mRNA-. Also, 16 out of 36 cases of WTS-PCR HPV DNA+ with multiple HPV infections were tested by LCM-PCR to determine whether one or more viruses are present in one lesion. 11 out of 16 (68.8%) multiple HPV infection cases were LCM-PCR HPV DNA- and were HPV mRNA-; 4 out of 16 (25%) were LCM-PCR HPV DNA+ and HPV mRNA+; Only 1 case was LCM-PCR HPV DNA+ and HPV mRNA-. In CADC, the kappa coefficient of RNAscope and WTS-PCR was 0.500 (p<0.001), while the kappa coefficient of RNA scope and LCM-PCR was 0.846 (p=0.001).

<table>
<thead>
<tr>
<th>Table 1. Concordance of HPV mRNA and DNA when using RNAscope and WTS-PCR</th>
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<tr>
<td>HPV DNA / HPV mRNA</td>
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<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
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</thead>
<tbody>
<tr>
<td>WTS-PCR (HPV DNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
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<td>16</td>
</tr>
<tr>
<td>Negative</td>
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<tr>
<td>Kappa coefficient</td>
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Table 2. Concordance of HPV mRNA and DNA when using RNAscope and LCM-PCR

<table>
<thead>
<tr>
<th>HPV DNA / HPV mRNA</th>
<th>RNA scope (HPV mRNA)</th>
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<tbody>
<tr>
<td>LCM-PCR (HPV DNA)</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Kappa coefficient</td>
<td>0.846 (P = 0.001)</td>
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**Conclusion**

Compared with WTS-PCR, using LCM-PCR for HPV DNA evaluation yielded lower prevalence of HPV DNA and better concordance of HPV mRNA and DNA when using RNAscope assay to evaluate the expression of HPV mRNA. Overall, HR-HPVs exist in CADC tissue with less active transcription, which implies that the causal role of HPV in CADC development need further study.
AN UPDATE ON THE INTERNATIONAL HPV REFERENCE CENTER

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Background / Objectives

To provide an update of ongoing activities at the International HPV Reference Center.

Methods

The center i) receives clones of potentially novel HPV types, clones them again and re-sequences them. If the confirmed sequence is found to represent a novel type, a unique HPV type number is assigned, reported to the originator and immediately posted on www.hpvcenter.se. ii) Distributes the reference clones, for academic research use, under Material Transfer Agreements agreed upon with the originator. iii) Provides a service with preliminary checking of whether new sequences may represent novel types. iv) issues international proficiency panels for HPV genotyping.

Results

Since 2013, 332 reference clones have been transferred to 55 different laboratories worldwide. Since the reference center was transferred from Heidelberg to Stockholm in 2012, 49 clones with putative new types have been submitted. Forty-six clones have been confirmed as novel types and assigned official numbers. One clone was found to be a subtype and two clones were not novel (recently established types, but not yet in GenBank). The g-genus now contains 90 HPV types, surpassing the diversity of the µ and β genera, which contain 65 and 51 HPV types, respectively.

Conclusion

Currently, the highest HPV type number awarded is HPV 216 (www.hpvcenter.se). Because 5 previously awarded HPV types have been withdrawn there are as of today (2017-04-27) 211 different HPV types.
DETECTION OF HPV mRNA AND HPV DNA UP TO 8 YEARS BEFORE DIAGNOSIS OF CIN3+

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Background / Objectives

Knowledge is sparse concerning proportions of normal cervical smears (Pap smears) that are positive for HPV mRNA and/or for HPV DNA among women who develop cervical intraepithelial neoplasia 3+ (CIN3+) or adenocarcinoma in situ (AIS). The aim is to compare proportions HPV mRNA- and HPV DNA-positivity of baseline cytology-samples before development of CIN3+ and AIS.

Methods

In Malmö of Sweden 2012 women were diagnosed with histopathology confirmed CIN3+ or AIS between 2007 through 2015, and also had a baseline biobank cytology sample at -80°C. Overall, 1204 cytology samples were eligible for HPV testing out of which 578 and 626 had normal and ASCUS+ cytology, respectively. The mean age at CIN3+ was 34 years (range 23-86) at diagnosis. The cell pellet from each LBC-sample (2mL SurePath solution) was suspended in 420 uL STM (Qiagen). Then a split sample approach was used where 100 uL was analyzed for high-risk HPV types by APTIMA (Hologic) and 100 uL by Cobas 480 (Roche).

Results

Among women with normal cytology 74% and 80% had HPV mRNA and HPV DNA, respectively. Among women with ASCUS+, 88% and 91% had HPV mRNA and HPV DNA, respectively. The overall agreement between the HPV mRNA and HPV DNA assays was 87% (K=0.64) and 92% (K=0.60) for those with normal and ASCUS+ cytology, respectively.

Among HPV-positive women with normal cytology, 8 years before diagnosis of CIN3+ 64% and 82% had detectable HPV mRNA and HPV DNA, respectively. Seven years before diagnosis: 85% vs 82%, 6 years: 69% vs 78%, 5 years: 73% vs 78%, 4 years: 73% vs 79% P=0.0108, 3 years: 78% vs 88%, 2 years: 79% vs 79%, 1 year: 58% vs 58%.

The median period between normal cytology and CIN3+ was 44 months (range 5-96) both for women with positive HPV mRNA-test and/or HPV DNA-test. Among HPV mRNA-positive (427 women) and/or HPV DNA-positive (460 women) cytologically normal women, 22% from each group had CIN3+ within 3 years.
The median period between ASCUS+ cytology and CIN3+ was 8 months (range 4 to 85) and 8 months (range 4 to 92) for women with positive HPV mRNA-test and/or HPV DNA-test, respectively.

Among 22 cases with histology of AIS, 95% (21/22) and 91% (20/22) had detectable HPV mRNA and HPV DNA in the cytology samples (all ASCUS+), respectively. The overall agreement between the HPV mRNA and HPV DNA assays was 95% (K=0.64).

Conclusion

High proportions of HPV positivity (74%-80%) were observed for HPV mRNA and HPV DNA assays among cytologically normal women who developed CIN3+. About a fifth of these HPV-positive cytologically normal women had diagnosis of CIN3+ within 3 years. Further studies are needed in order to predict which of HPV-infected women who will progress to high-grade dysplasia.
FC 05-09
HPV DNA GENOTYPE AGREEMENT AND CLINICAL PERFORMANCE IN FIRST-VOID URINE AND CERVICAL SAMPLES IN A REFERRAL POPULATION IN BELGIUM

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Background / Objectives

This study reports on the performance and acceptability of optimized HPV detection and genotyping in self-collected first-void urine (FvU) versus cervical (Cx) samples for the detection of high-risk (hr)HPV DNA and cervical precancerous lesions in a referral population in Belgium.

Methods

Women between 25-64 years old (median age: 36.00 ± 10.08 year) referred for colposcopy at the Antwerp University Hospital (UZA) collected a FvU sample (Colli-PeeTM, Novosanis) (NCT02714127) prior to their visit with the gynecologist. HPV DNA genotyping was performed on paired FvU (after in-house DNA extraction (1)) and Cervex-Brush® (Rovers Medical Devices) collected Cx samples in PreservCyt® (Hologic) with the Riatol qPCR HPV genotyping assay (Belgium) (2). Histology on biopsies (when indicated) was investigated at the pathology laboratory (UZA). Data regarding acceptability of different sampling methods were gathered through questionnaires. Statistics was performed using IBM SPSS24.

Results

HrHPV DNA was detected in 69.09 (n=76/110) and 66.36% (n=73/110) of FvU and Cx samples respectively, with HPV16 the most prevalent genotype (n=23/110 versus n=21/110). A good agreement for hrHPV DNA in FvU and Cx samples of 86.36% (Cohen’s kappa: 0.688; 95% CI: 0.543-0.833) was found. On individual genotype level, excellent agreement for HPV16 (96.36%; Cohen’s kappa: 0.886; 95% CI: 0.776-0.996) and HPV18 (99.09%; Cohen’s kappa: 0.918; 95% CI: 0.759-1.077) was
obtained. Moreover, significant positive correlations of HPV16 and 18 DNA copies per µl DNA extract were found between both sample types (Spearman rho HPV16: 0.570, p-value: 0.009; and HPV18: 0.829, p-value: 0.042). For 33 out of 110 samples with histological reference, a relative sensitivity for CIN2+/CIN3+ and specificity for <CIN2 of HPV16/18 detection in FvU versus Cx samples of 1.00 (both CIN2+ and CIN3+) and 0.93 (<CIN2) was acquired.

Preference of using a female urination device upon FvU collection compared to use of a standard urine cup, a pap smear taken by a clinician, or use of a vaginal self-sampling device was respectively 91.75 (n=89/97), 77.36 (n=82/106), and 91.67% (n=11/12).

Conclusion

Good to excellent agreement between FvU and Cx samples on hrHPV DNA and HPV16/18 genotype level was obtained, with significant positive correlations in HPV16/18 copies per µl DNA between both samples. These results furthermore demonstrate that FvU self-sampling is highly preferred among 25-64 year old women referred to colposcopy in Belgium.

References

ANTIRETROVIRAL THERAPY, HIGH-RISK HUMAN PAPILLOMAVIRUS AND CERVICAL INTRAEPITHELIAL NEOPLASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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1 London School of Hygiene and Tropical Medicine (United kingdom), 2 Catalan Institute of Oncology (Spain), 3 CIBER en Epidemiología y Salud Pública (Spain)

Background / Objectives

The interactions of antiretroviral therapy (ART) with high-risk (HR) HPV and cervical lesions in women living with HIV (WLHIV) are poorly understood. We reviewed HR-HPV and cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesions (SIL) outcomes in ART-taking compared to ART-naive WLHIV.

Methods

We performed a systematic review and meta-analysis by searching Medline and Embase databases for cross-sectional or cohort studies from 1 January 1996 to 6 November 2016 that reported the association of ART with prevalence of HR-HPV or prevalence, incidence, progression or regression of CIN or SIL abnormalities. We performed random-effects meta-analyses to estimate summary statistics. Heterogeneity was examined using the I2 statistic.

Results

A total of 6,441 and 8,262 WLHIV were included from 29 studies evaluating the association of ART with prevalence of HR-HPV and high-grade CIN (CIN2+) or SIL (HSIL+), respectively. ART users had lower HR-HPV prevalence than ART-naive WLHIV (adjusted Odds Ratio [aOR] =0.83, 95%CI: 0.70-0.99, I2=51%, adjusted for CD4+ count and ART duration), and was also lower among prolonged ART users (>2 years) compared to short-duration users and ART-naive combined (crude OR=0.65, 95%CI: 0.55-0.77, I2=0.0%). There was some evidence of lower risk of CIN2+/HSIL+ among ART users (aOR=0.65, 95%CI: 0.40-1.06, I2=30%).

Sixteen studies reported the association of ART with longitudinal cervical lesions (SIL) outcomes, from a combined total of 6,664 WLHIV. ART use was associated with a lower risk of any SIL incidence (adjusted Hazard Ratio [aHR] =0.64, 95%CI: 0.47-0.86, I2=19%, adjusted for time-varying ART and CD4+ count), and progression (aHR=0.64, 95%CI: 0.54-0.75, I2=18%) and increased likelihood of SIL regression (aHR=1.58, 95%CI: 1.28-1.94, I2=18%).

Conclusion
Prolonged ART use in WLHIV can decrease the risk of HR-HPV and CIN2+/HSIL+ prevalence, SIL incidence and progression and induces regression.
INCIDENCE TRENDS IN HPV-RELATED CANCERS IN NORWAY, AND CASES PREVENTABLE BY HPV VACCINATION

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Cancer Registry of Norway (Norway)

Background / Objectives

To examine incidence trends in squamous cell cancers of the cervix, vulva, vagina, anus, penis and oropharynx, and cervical adenocarcinoma for the period 1953-2015, and to determine how many currently incident cases may be prevented by bi/quadrivalent and nona-valent HPV vaccination

Methods

We extract data from the Cancer Registry of Norway, which has complete and accurate registration of all cancer cases in Norway for the whole study period. Trends in incidence are examined by joinpoint regression and the annual percentage change statistic for each cancer site and for each sex. To estimate the preventive potential of HPV vaccination, we use previously published accounts of fractions attributable to HPV for each cancer site

Conclusion

Over the period 1953-2015, we observe significantly increasing incidences of anal, oropharyngeal, penile and vulvar cancer, and of cervical adenocarcinoma. The increase was most pronounced, with annual percentage changes exceeding 2, for anal cancer (for each sex) and for oropharyngeal cancer among men. Cervical squamous cell cancer incidence decreased after the introduction of screening, but remained stable after 2004. The incidence trends highlight the importance of primary prevention of HPV-related cancers. We show that the number of cases that can be prevented by HPV vaccination in Norway is substantial, also for non-cervical HPV-related cancers, and among men. Moreover, in comparison with the bi/quadrivalent HPV vaccines, use of the nona-valent vaccine will prevent a substantial additional number of cervical cancer cases in Norway
FC 06-03
A COMPREHENSIVE LANDSCAPE OF 27 HPV VIRUSES’ PREVALENCE AND MULTI-INFECTION PATTERNS, HIGH CONSISTENCY BETWEEN THE HPV16/18 CO-INFECTION PREFERENCE PATTERN AND THE CROSS-PROTECTIVE EFFICACY OF HPV16/18 VACCINE AGAINST NON-VACCINE HPV TYPES.


1 National Cervical Cancer Consortium of China (China), 2 Cancer Hospital Chinese Academy of Medical Sciences (China), 3 The 1st Affiliated Hospital, Wenzhou Medical School (China), 4 Xijing Hospital, The Fourth Military Medical University (China), 5 West China Second University Hospital (China), 6 Changning Maternity and infant Health Hospital (China), 7 The 1st Affiliated Hospital of University of South China (China), 8 General Hospital of Beijing Aerospace (China), 9 Suzhou Municipal Hospital (China), 10 Sichuan University (China)

Background / Objectives

In China, the attribution of HPV52/58 is significantly higher than elsewhere. China’s medical resources are concentrated in tertiary hospitals, where bearing “HPV-heavy-burden”, which can provide representative samples to delineates a national comprehensive landscape of the prevalence and multi-infection patterns of HPV among gynecological outpatients (GOP), and to evaluate the cross-protective efficacy of HPV16/18 vaccine.

Methods

We recruited participants out of GOP from 8 tertiary hospitals in 7 provinces of China. Cervical exfoliated cell samples were collected for HPV genotyping using Tellgenplex™ HPV DNA Assay. Odds ratio was used for the evaluation of the preference of any two types co-infection (AB): the real infection rate of AB divided by its theoretical rate (the multiplication of the single infection rate of A and B).

Results

Among 137,949 samples from GOP, the total prevalence of 27 HPVs (17hr/10lr) was 23.5%. Age-specific prevalence showed a flat “U-formed” pattern. The most prevalent hrHPVs were all from α9:16(3.3%), 52 (2.3%), 58 (1.9%). The most prevalent lrHPVs were both from α3: 81(0.9%), 61(0.9%). Overall, the prevalence of
6 and 11 were 0.6% and 0.3%, which differed by geographic region and decreased with age. Multi-infection was identified in 25.8%. The two-types-infection was predominant. We found that 15% of hrHPV infections were co-infected with lrHPV; while 40% lrHPV infections were co-infected with hrHPV. HPV16 consisted of 66.51% single infection, the highest, followed by 52(60.2%), 58(59.81%), while 26(33.3%) as the lowest. The mixed genotypes 16+58(283) and 16+52(265), 52+58(242), 16+18(195) were the most common multi-infections. The co-infection (AB) preference pattern of 13 hrHPVs to 16 was 31(3.4), 45(2.8), 33(2.7), 35(2.4), 18(2.3), 56(2.0), 59(1.8), 58(1.7), 39(1.7), 66(1.7), 51(1.6), 68(1.3), 52(1.2). The co-infection (AB) preference pattern of 13 hrHPVs to 18 was 31(3.9), 35(3.0), 56(2.7), 51(2.7), 33(2.6), 66(2.5), 59(2.4), 58(1.6), 39(2.2), 45(2.0), 52(1.7), 58(1.6), 68(0.7). This analysis revealed that HPV31 was the most involved in HPV 16/18 co-infection, while HPV52/58 had less co-infection preference to HPV16/18.

Conclusion

These co-infection (AB) preference patterns are highly consistent with cross-protective efficacy of HPV16/18 vaccine against HPV31, but almost negative vaccine efficacy against HPV52/58. On one side, this finding may explore the mechanism of cross-protection of HPV vaccines. On another side, it indicates that it is urgently needed to evaluate the efficacy of HPV vaccines and the influences to the HPV epidemic in China, where with high prevalence of HPV52/58 as the current vaccines only covering HPV16/18.

References

1) Co-infection with HPV types from the same species provides natural cross-protection from progression to cervical cancer. Sobota et al.. Infectious Agents and Cancer 2014, 9:26

2) Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Wheeler et al.. Lancet Oncol 2012; 13: 100–10


ESTIMATION OF THE OVERALL BURDEN OF CANCERS, PRECANCEROUS LESIONS, AND GENITAL WARTS ATTRIBUTABLE TO 9-VALENT HPV VACCINE TYPES IN WOMEN AND MEN IN EUROPE

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Background / Objectives

In addition to cervical cancer, human papillomavirus (HPV) is responsible for a significant proportion of cancers and precancerous lesions of the vulva, vagina, anus, penis, head and neck, as well as genital warts. We estimated the annual number of new cases of these diseases attributable to 9-valent HPV vaccine types in women and men in Europe.

Methods

The annual number of new cancers of the cervix, vulva, vagina, anus, penis, and selected head and neck sites in the population of the European Medicines Agency territory was estimated based on age-specific incidence rates extracted from Cancer Incidence in 5 Continents, Volume X and Eurostat population data for 2015. The annual number of new cancers attributable to 9-valent HPV vaccine types was estimated by applying the HPV attributable fraction from reference publications based on a large European multicenter study. For non-cervical cancers, HPV attributable fractions were based on oncogenically-active HPV infections only (i.e., detection of HPV DNA and either mRNA and/or p16 positivity). For precancerous lesions of the cervix, vulva, vagina, and anus, and for genital warts, previously published estimations were updated for the 2015 population.

Results

The annual number of new cancers attributable to 9-valent HPV vaccine types was estimated at 47,992 (95% bound: 39,785-58,511). Cervical cancer showed the highest burden (31,130 cases), followed by head and neck cancer (6,786 cases), anal cancer (6,137 cases), vulvar cancer (1,466 cases), vaginal cancer (1,360 cases), and penile cancer (1,113 cases). About 81% were estimated to occur in women and 19% in men. The annual number of new precancerous lesions (CIN2+, VIN2/3, VaIN2/3, and AIN2/3) and genital warts attributable to 9-valent HPV vaccine types was estimated at 232,103 to 442,347 and 680,344 to 844,391, respectively.
Conclusion

The burden of cancers associated with 9-valent HPV vaccine types in Europe is substantial in both sexes. Head and neck cancers constitute a heavy burden, particularly in men. Overall, about 90% of HPV-related cancers, 80% of precancerous lesions, and 90% of genital warts are expected to be attributable to 9-valent HPV vaccine types each year, demonstrating the important preventive potential of the 9-valent HPV vaccine in Europe.
Declines in genital warts diagnoses since change in 2012 to use the quadrivalent HPV vaccine in England: data to end 2016

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Background / Objectives

A national school-based HPV vaccination programme for girls aged 12-13 years old was introduced in the UK in September 2008 offering the bivalent HPV 16/18 vaccine. In 2012, the programme changed to offer the quadrivalent vaccine, additionally protecting against HPV types 6 and 11, responsible for approximately 90% of genital warts (GW). Coverage for the vaccination programme has been high, with over 85% of routine cohorts completing all doses. Previously reported data have shown modest declines in GW diagnoses, suggesting a potentially cross-protective effect of the bivalent vaccine against GW. We present the first evidence of declines in GW diagnoses following the programme change to the quadrivalent vaccine.

Methods

Data were obtained from the GUM Clinic Activity Dataset (GUMCADv2) submitted by GUM and integrated GUM/sexual and reproductive health clinics for years 2009-2016. GUMCADv2 is a mandatory reporting system, providing disaggregate records of all attendances, testing and diagnoses at GUM clinics in England and has been reported to Public Health England (PHE) since 2008, with full coverage from 2009. All records coded as first episode GW for females and males aged 15-24 years old were extracted. Diagnoses of recurrent GW were excluded.

Results

Data to end 2015 – available at abstract submission: In 2015, 254,775 and 77,584 attendances were recorded by GUMCADv2 in 15-19 year old females and males, respectively. The rate of GW diagnoses for females aged 15 to 19 years was 38.9% lower (from 685.8 to 419.2 per 100,000 population) in 2015 than in 2009, and 30.2% lower (from 274 to 191.2 per 100,000 population) for 15-19 year old males. Over the same time period, the greatest declines were observed in 15 year old females (83.2%) and 16 year old females (58.0%); around 2/3 of vaccinated 15 year olds and 1/6 of vaccinated 16 year olds would have received the quadrivalent vaccine. Reductions in the rate of GW diagnoses among same aged males were 31.6% and 32.7%. Decreases of 17.5% (from 698.9 to 576.8 per 100,000 population) and 15.5% (from 849.6 to 718.2 per 100,000 population) were seen in 20-24 year old females and males, respectively.

Conclusion
The moderate, unexpected declines in GW that we have seen since the introduction of a high coverage HPV vaccination programme using the bivalent vaccine are being followed, as expected, by much larger declines amongst females offered the quadrivalent vaccine.

These ecological observations suggest that the high coverage female-only HPV vaccination programme is affording substantial herd protection to young males.

We will present analyses including data to end 2016 (available in June 2017), with additional sub-group analyses.
CHARACTERIZATION OF GENOTYPE-SPECIFIC HPV PREVALENCE IN CUTANEOUS WART BIOPSIES

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Background / Objectives

Cutaneous warts are a common, infectious and sometimes very painful problem, with a varying worldwide prevalence of 0.84-12.9%1. Warts are caused by infection with the human papillomavirus (HPV). The most frequently found HPV types in cutaneous warts are HPV1, 2, 3, 4, 7, 10, 27, 41, 57, 60, 63 and 65. The aim of this study was to develop a high-yield DNA extraction protocol for formalin-fixed paraffin-embedded biopsies and determine the prevalence of HPV in cutaneous warts in a Belgian population.

Methods

A total of 50 biopsies were included in this study. Before and after slicing of the sections predetermined for DNA extraction (10x5µm), additional sections were made for haematoxylin-eosin (HE) staining to ensure that these were derived from wart epithelium. The optimized protocol involved overnight Proteinase K and EDTA digestion, followed by automated extraction on the NucliSENS® easyMAG® system (bioMérieux). A newly developed wart-associated HPV qPCR assay, capable of detecting the above mentioned cutaneous types, together with the in-house HPV Riatol genotyping assay, capable of detecting the most relevant mucosal types (HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67 and 68), were used to determine the HPV prevalence.

Results

The wart diagnosis was confirmed by HE staining. All samples tested positive for B-globin (cell control) and were considered valid. 24% [95%CI 12-36%] of the samples were negative for the above mentioned cutaneous as well as mucosal HPV types. Cutaneous HPV types 41, 60 and 63 were not detected. 8% [95%CI 0.5-16%] of the samples was infected with mucosal low-risk (HPV6 and 11) and high-risk (HPV16, 58 and 59) HPV types and 36% [95%CI 23-49%] contained multiple infections.

Conclusion
The most prevalent HPV types in the Belgian population were HPV1, 57, 4, 2, 27 and 7. Multiple HPV infections were detected in 36% of lesions, contradicting the current literature claiming that in immunocompetent patients only 0-16% of cutaneous warts exhibit multiple HPV infections\textsuperscript{2,3,4}. Considering that cutaneous warts are very inconvenient disorders that can cause not only pain, but also diminish the quality of life of the affected individuals, a more efficient management should be implemented. Since it has been suggested that HPV type can influence natural course and response to treatment in certain subsets of verrucae\textsuperscript{2}, genotype specific strategies should be considered, indicating an important role for future HPV genotyping.

References


BURDEN OF GENITAL WARTS IN PERU, ARGENTINA AND ECUADOR: AN OBSERVATIONAL STUDY

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Background / Objectives

Genital warts (GW) are mucosal or skin lesions caused by human papilloma virus. The burden of disease due to genital warts in Latin America is not well characterized. The study objectives were to estimate the burden of genital warts (GW) within the healthcare system and usual practices of GW management in Peru, Argentina and Ecuador.

Methods

We recruited a convenience sample of 250 physicians from the public sector in Peru and both the public and private sector in Ecuador and Argentina: primary care (28), gynecology (119), urology (30), dermatology (50), infectious diseases (10), proctologists (2) and other (11). Physicians completed a daily log of all patients 18-60 years of age seen over 10 days in their offices, as well as a survey collecting data on patient demographics, GW diagnosis, referral patterns, in-office procedures, duration of treatment and estimated number of office visits required for treatment.

Results

The 250 physicians reported seeing a total number of 31,111 patients, 77.1% were women. 1,294 males and females had a GW diagnosis, 38.02% were in men. GW overall prevalence was 4.16% (95% CI 3.94% - 4.39%); 2.28% (95% CI 2.02-2.56) in Peru, 5.51% (95%CI 5.10-5.92) in Ecuador and 5.1% (95%CI 4.54-5.58) in Argentina. The prevalence was highest among primary care providers in Peru, 4.68 (98% CI 3.86-5.59), infectious disease specialists in Ecuador 7.38 (95%CI 4.53-10.23) and urologists in Argentina 10.9% (95% CI 8.9-12.8). Of the GW cases observed, 52.7% were the first reported episode in the patient’s life, 12.5% were cases without an episode in the previous 12 months and 34.8% were existing cases. Peru reported the highest proportion of first time cases, 64.0%, with Ecuador and Argentina reporting 50.4% and 48.2% respectively. Most physicians reported seeing patients who were direct-consult.
Conclusion

GW cases are commonly seen by physicians in Peru, Ecuador and Argentina and only a slight majority (52.7%) of these was the first reported episode in the patient’s life. In Peru, cases were most often seen in primary care providers, whereas in Ecuador and Argentina cases were seen by specialists, with that being said, physicians recruited in Peru were from the public sector while those in Ecuador and Argentina were a mix of public and private providers. Our data suggests that GW may represent a substantial healthcare burden in Peru, Argentina and Ecuador.
AN OVERVIEW OF CERVICAL CANCER EPIDEMIOLOGY AND PREVENTION IN SCANDINAVIA

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Background / Objectives

In the Scandinavian countries of Denmark, Norway and Sweden, organised cervical cancer prevention programmes have contributed to reducing the cervical cancer burden. However, new technologies, such as primary human papillomavirus (HPV) DNA testing and HPV vaccination, necessitate comprehensive policy analyses to identify optimal prevention approaches. To inform future policy analyses, we aimed to provide an overview of cervical cancer epidemiology and existing prevention efforts in Scandinavia.

Methods

We compiled and summarised data on current prevention strategies, population demography, and epidemiology for each Scandinavian country by reviewing published literature and official guidelines, performing registry-based analyses using primary data, and discussions with experts in each country. We compared age-specific cervical cancer incidence for years 1960-66 and 2010-14 across the countries using Poisson regression with indicators for five-year age-groups (ages 20-84 years) and for each country. We also assessed country-specific variations in age-specific HPV prevalence using Fisher’s exact test and logistic regression.

Results

In general, nationwide organised cytology-based screening was implemented in all Scandinavian countries by 1996, but opportunistic screening occurred as early as the 1950s. Prior to implementation of widespread screening and during years 1960-1966, cervical cancer incidence was considerably higher in Denmark than in Norway and Sweden. Decades of cytology-based screening later (i.e. years 2010-14), the incidence remains the lowest in Sweden, with Norway and Denmark having an age-adjusted incidence rate ratio (95% CI) of 1.28 (1.20-1.37) and 1.36 (1.28-1.45), respectively. HPV prevalence peaks at younger ages (i.e. younger than age 24
years) and thereafter decreases by age for all genotypes in all countries, but was generally lowest in Sweden. For all countries the most prevalent HPV genotypes were HPV16, 18 and 31.

Conclusion

Scandinavian countries generally face similar cervical cancer burden and utilise similar prevention approaches; however, important differences remain as cervical incidence and HPV prevalence remains lowest in Sweden. Future policy analysis will need to evaluate whether these differences warrant differential prevention policies, or whether efforts can be streamlined across Scandinavia.
Type-specific human papillomavirus profile, absolute risk and attributable fraction to cervical cancer and precancerous lesions—a population-based study of 3,083 women in Inner Mongolia, China.

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Background / Objectives

Given the variation of region-specific HPV genotypes prevalence, knowledge about the distribution of human papillomavirus (HPV) genotypes in general population and their attribution to high-grade cervical lesions is crucial to guide the introduction of prophylactic vaccines and the implementation of cervical cancer screening. Few studies, however, comprehensively focused on the HPV genotypes profiles in such high-risk regions with ethnic minority as Inner Mongolia, in China. To analyze the HPV genotypes characteristics and the presence of multiple HPV infections among general population and estimate type-specific absolute risk and relative contributions to cervical intraepithelial neoplasia grade 2 or worse (CIN2+).

Methods

Between Jun and Aug 2016, 3,083 women aged 21–64 years were enrolled in a cervical cancer screening study in Inner Mongolia Province, China. Each participant was examined by Hybrid Capture 2 testing and liquid-based cytology. Women positive with any screening results were referred to colposcopy and biopsy was taken if necessary. All cervical cytological cells were tested using SPF10-LIPA system to discriminate 28 HPV genotypes. Absolute risk of cervical (CIN2+) for type-specific HPV was calculated and the corresponding attribution to cervical lesions was estimated using a fractional contribution approach.

Results

High-risk HPV (HR-HPV) prevalence was 17.5% and abnormal cytology rate was 14.2% in the general population. Most five common genotypes were HPV 52, 39, 16, 51, 58. Multiple-type infection rate varied by age and peaked at women with 50 years old and more (12.0%). Women infected with HPV16 were at highest absolute risk of CIN2+ at 28.7%, followed by HPVs 58, 33, 35, 18 and 52 at 12.0%, 11.8%, 9.5% and...
9.3%, 8.8% respectively. Attributable fraction (AF) to CIN2+ differed by type-specific HPV and was predominated by HPV 16 with the AF of 54.9%, followed by HPV 52 (52.4%), HPV 39 (4.6%), HPV 58 (2.7%), HPV18 (1.9%).

**Conclusion**

Type-specific high-risk HPV-DNA-based screening tests and protocols and introduction of polyvalent HPV vaccines might give the priority to HPV types 16, 18, 52 and 58 in the high-risk region in China, as their high prevalence, high absolute risk and notable attributable fraction to high-grade cervical lesions.
SEX DIFFERENCES IN PREVALENCE, INCIDENCE AND CLEARANCE OF ANOGENITAL HUMAN PAPILLOMAVIRUS INFECTION IN CHINA: A POPULATION-BASED PROSPECTIVE STUDY

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Background / Objectives

Understanding sex differences in natural history of anogenital human papillomavirus (HPV) infection is essential for making policies to prevent and control HPV infection and related diseases. However, there is a scarcity of researches focused on both sexes, and direct comparing among different studies is difficult due to different sampling and typing methods.

Methods

From May to July 2014, a total of 2309 men and 2378 women aged 18-55 years old were enrolled from communities and universities in Liuzhou, China. Penis / glans penis / coronary sulcus and perianal / anal canal (PA) specimens of men and vaginal, vulvar and PA specimens of women were collected biannually for up to three visits and genotyped for 12 oncogenic HPV (classified as Group 1 by IARC) and 2 non-oncogenic HPV types (HPV 6 and 11) by PCR. Prevalence analysis was performed among 1937 (83.9%) men and 2344 (98.6%) women with the valid HPV typing result at baseline. Totally 1643 (71.2%) men and 1752 (73.3%) women with a median follow-up of 12.5 months (range 5.0-19.1) and 12.6 months (range 5.0-20.1), respectively, were included in incidence analysis.

Results

The prevalence of oncogenic HPV type was higher in women than that in men (18.7% vs 9.4%, P < .001), whereas the prevalence of HPV 6 and 11 infection was similar (1.4% vs 1.2%, P = .6832). Incidences of oncogenic HPV infection in men and women were 10.1 (95% confidence interval (CI) 8.6-11.5) and 9.4 (95% CI 8.2-10.6) per 1000 person-months, respectively, and no sex differences were found (P = .4659). However, men was more likely to acquire HPV 6 or 11 infection than was women (2.0 vs 1.1, P = .0223). Median duration of HPV infection was longer in women than that in men for both oncogenic (11.6 months vs 6.8 months, P < .001) and non-oncogenic (11.9 months vs 6.4 months, P < .001) types. Both prevalence and incidence of oncogenic HPV infection decreased with age in women, but did not
vary by age in men. Besides sex behavior, hygiene behavior was also associated with prevalence and incidence of HPV infection in both sexes.

Conclusion

This is the first large population-based prospective study focused on HPV prevalence, incidence and clearance in anogenital sites of both sexes. For oncogenic HPV, though newly acquired anogenital infections were comparable between men and women, the median duration of infection was shorter in men, thus women were more of a major reservoir than men. For HPV types 6 and 11, men had a higher speed to both acquire and clear infection, thus the two sexes seem to contribute similarly to the virus circulation. Our study indicated that interaction of host and virus might be different for oncogenic and non-oncogenic HPV types between sexes.
EVALUATION OF MUCOSAL AND SYSTEMIC IMMUNOGLOBULIN A/G RESPONSES ONE YEAR AFTER 3 DOSES OF THE HUMAN PAPILLOMAVIRUS-16/18 ASO4-ADJUVANTED VACCINE

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Background / Objectives

Vaccination against oncogenic human papillomavirus (HPV) types is an intervention for cervical cancer prevention. Despite there being a relatively long period of time since the beginning of clinical use of HPV vaccines no evidence-base data is available on the need of a boost vaccination. We investigated one-year post-vaccination antibody responses against HPV 16/18 by detection of IgG and IgA HPV-specific antibodies in cervical secretion samples and serum.

Methods

This study was designed to describe the course of IgG/IgA responses in cervical secretions and in serum one year after the first dose of intramuscular administration of the HPV16/18 AS04-adjuvant vaccine. Blood and cervical mucus samples were collected for immunologic assays, 7 months after the first doses and 1 year following the last boost vaccination (month 7) by enzyme linked immunosorbent assay (ELISA). The detection of IgG and IgA anti-HPV/VLP was developed for this purpose.

Results

It was observed that approximately 100% of the IgG serum samples reacted when the antigen was present in the first dilution of both collections. The positivity, however, decreases, according to the dilutions. Regarding IgA reactivity in serum, initial conversion was observed in 95% at month 7 of vaccination and 79% after 1 year. Similar results can be seen in the mucus samples with a higher positivity at month 7 and decreasing after 1 year, lower levels of IgG and IgA antibody were detected in the cervical mucus (33%) and 29%, respectively, after 1 year of vaccination. The median absorbance detected in serum samples for IgG and IgA anti-HPV-VLP antibodies was significantly higher at 7 months after vaccination, in the dilutions 1:100, 1:1.000, 1:10.000 and 1:10, respectively, when compared to 1 year after vaccination (P<0.0001). The median absorbance detected in cervical mucus samples was significantly higher at 7 months after vaccination, in the dilutions 1:100, 1:1.000 and 1:10 for anti-HPV-VLP IgG and IgA, respectively, when compared to 1 year after, with 1:100 and 1:10, for IgG and IgA respectively (P< 0.0001). In
serum and cervical mucus samples, the median absorbance was significantly higher 7 months after vaccination and it is possible to see the decrease at 1 year after, according to dilutions.

**Conclusion**

One year after the first dose, the immune responses induced by the HPV-16/18 AS04-adjuvant vaccine were significantly decreased in cervical secretion samples and serum when compared to seven months after the first dose. A possible vaccine booster may be necessary. However, longer follow-up studies are necessary to assess the need for booster doses after primary vaccination with 2 as well as with 3 doses.
ADVANCING HPV VACCINE DELIVERY: 12 PRIORITY RESEARCH GAPS


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Background / Objectives

Recent reviews have identified interventions for increasing HPV vaccination, but effects were small and evidence was often insufficient to identify best practices. The National HPV Vaccination Roundtable sponsored a one-day meeting in the United States in 2016 on best and promising practices in HPV vaccine delivery, in part to identify important research gaps.

Methods

Meeting participants were ~500 HPV vaccine delivery experts including scientists, clinicians, and other stakeholders, including ~400 who streamed the event online. Throughout the meeting, facilitators encouraged attendees to identify gaps that future research should address, and write them on display boards or send via email or Twitter. In-person attendees then voted for up to five gaps they believed were top priorities. Gap numbers refer to their priority ranking, with Gap 1 having received the most votes.

Results

Attendees identified 33 research gaps. Several themes emerged among the 12 prioritized gaps. One theme was social media and vaccine confidence, which included: Gap 1, how to increase HPV vaccine confidence by intervening in social media; Gap 4, how to address rumors about HPV vaccine that spread via social media; and Gap 8, how to address parents’ concerns and hesitancy about HPV vaccine. A second theme was healthcare provider interventions, which included: Gap 2, how to encourage providers to attend in-clinic quality improvement interventions; Gap 6, how to intervene with the entire medical team; and Gap 10, how to increase HPV vaccination during acute care visits. A third theme was system-level approaches, which included: Gap 3, best practices for health insurers and plans; Gap 12, the impact of quality standards; Gap 11, effective system-level changes in large health systems and hospitals; and Gap 5, the impact of connecting immunization information systems to electronic health records (EHRs) and exchanging data bidirectionally. Two other prioritized gaps that did not fit these themes were Gap 7,
determining what interventions work in rural areas; and Gap 9, the impact of survivor testimonials.

Conclusion

Experts identified and prioritized research gaps that may have promise for increasing HPV vaccination in the US and internationally. It is critical to develop and evaluate interventions in each of these areas to close existing gaps and identify best practices for increasing HPV vaccination. Grant support: US Centers for Disease Control and Prevention (1H23IP000931-01, Saslow, PI).
TRENDS IN PREVALENCE OF HUMAN PAPILLOMAVIRUS TYPES AND THE IMPACT OF NONA VALENT VACCINATION: ANALYSIS ON 13,665 PATIENTS OVER A 18-YEAR STUDY PERIOD

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Background / Objectives

Human Papillomavirus, HPV, vaccination significantly reduced the incidence of cancerous/precancerous condition of the genital tract. The quadrivalent vaccine type 6,11,16,18 was licensed in 2006; more recently, the Food and Drugs Administration (FDA) approved the nonavalent vaccination against HPV. Here, we aimed to test the theoretical utility of the incorporation of nonavalent vaccination into a clinical setting.

Methods

Data of consecutive patients undergoing sampling for HPV DNA testing from 1998 to 2015 were retrospectively searched in order to identify changes in HPV prevalence during three study periods (T1, 1998-2003; T2, 2004-2009; and T3, 2010-2015).

Results

We enrolled 13,665 patients: 1361, 5130, 7174 patients, in T1, T2 and T3, respectively. Potentially, the quadrivalent vaccine protected against HPV infection in 71.5%, 46.5% and 26.5% of patients tested in T1, T2 and T3, respectively (p-trend<.001). While, the nonavalent vaccine protected against HPV infection in 92.5%, 72.3% and 58.1% of patients tested in T1, T2 and T3, respectively (p-trend<.001). The proportion of patients with genital dysplasia grade 2+, not related to HPV genotypes covered by quadrivalent vaccine (13% in T1, 21% in T2 and 34% in T3) and nonavalent vaccine (3% in T1, 12% in T2 and 19% in T3) increased over the time (p-for-trend<.001). For all study period the nonavalent vaccine was superior that quadrivalent vaccine in protect against HPV infection (p<.001).

Conclusion

Our data suggested that potentially the introduction of the nonavalent vaccine would improve protection against HPV infections and HPV-related genital dysplasia 2+. Moreover, we can speculate that cross protection of nonavalent vaccine will be related to a highest coverage against other HPV types.
FC 07-04
DESIGN, BASELINE FINDINGS AND HPV GENOTYPES FROM A RANDOMIZED CONTROLLED TRIAL WITH THE QUADRIVALENT HPV VACCINE COMPARING A 2-DOSE (0, 6 MONTHS) TO AN EXTENDED (0, 6, 60 MONTHS) SCHEDULE: ICI-VPH STUDY

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Background / Objectives

In Quebec (Canada), the HPV vaccination program targets 9-10 year-old girls who receive 2 doses of the quadrivalent HPV vaccine (Q-HPV) at 0, 6 months, through a school-based program. The main objectives of ICI-VPH are to evaluate whether a 2-dose schedule (0, 6 months) is non-inferior to a 3-dose schedule (0, 6, 60 months) to prevent persistent HPV 16 and 18 infections and to compare HPV6-11-16-18 GMTs between the 2 study groups, 10 years after the first dose. Here, we present the design of the trial, the baseline characteristics of the participants, and the HPV genotypes identified at recruitment.

Methods

We recruited teenage girls who had been vaccinated with 2 doses of Q-HPV in fourth grade, 5 years earlier. The participants were randomized to receive a 3rd dose or not. Participants self-collected a vaginal sample for HPV genotyping at baseline and will do so every 6 months for 5 years. Participants also provided health and behaviour data at baseline and will continue to do so every year for 5 years. We collected a blood sample (first 500 participants) at baseline for HPV serology testing, and other samples will be collected after 2.5 years and 5 years. Self-collected vaginal swabs are tested for HPV by a generic HPV PCR assay, and if positive, tested with the Linear Array for the detection of 36 genotypes.

Results

Between 2013 and 2016, we randomized 3364 participants 13-16 years of age, in the province of Quebec, Canada. The 2 study groups were comparable at recruitment:
92.2% were born in Canada, 85.4% identified as French Canadians, 91.0% were non-smokers, 16.0% were sexually active and 21.2% had used hormonal contraception. Among those reporting sexual activity, 80.5% had had intercourse. Genotyping results at recruitment were available for 216 participants who reported having had intercourse. Among them, HPV prevalence was 8.3% (n=18). Single infections (4.2%) were as frequent as multiple infections (4.2%). The most prevalent types were: HPV 51 and HPV 84 (each 2.8%, n=5); HPV 62 (1.9%, n=4); HPV 53, HPV 66, HPV 73, HPV 89 (each 1.4%, n=3); HPV 33, HPV 56 (each 0.9%, n=2); HPV 31, HPV 35, HPV 39, HPV 40, HPV 42, HPV 58, HPV 59, HPV 67 (each 0.5%, n=1). HPV types 6/11/16/18 were not detected.

**Conclusion**

Vaccine targeted types were not detected in this cohort of 13-16 year-old, 5 years after vaccination with a 2-dose schedule of Q-HPV. To our knowledge, this ongoing study is the first to assess the role of an HPV booster dose for the Q-HPV vaccine within a high-coverage vaccination program.
Efficacy of HPV vaccine in young women in Colombia after five years of its introduction.


Background / Objectives

Reduction in the prevalence of vaccine type HPV infection in young women offers the opportunity to monitor early effects of vaccination program. In Colombia, HPV vaccination was introduced in 2012 by National Immunization Program as primary strategy for the prevention of cervical cancer. We evaluated the program’s impact on genotype-specific HPV infection prevalence and the distribution in a group of non-vaccinated vs vaccinated young women aged 18-25 years old from Colombian

Methods

Young women aged 18–25 years from Manizales were invited to participate to this study through different communications strategies established at local health centers and Technological and higher education institutes. Cervical samples were tested for type-specific HPV DNA using a Linear Array genotyping test. HPV prevalence infection among 807 women in the post-vaccine group (2016–2017) were compared with prevalence of 951 women non-vaccinated (2015).

Results

The prevalence of vaccine HPV genotypes (16, and 18) was significantly lower in vaccinated sample than in the non-vaccinated sample: 4.8 % (39/807) vs 15.5% (147/951); P < .001. Moreover, this reduction was higher in women vaccinated before starting sexual activity compared to after 2.4% (8/329) vs 6.5% (31/478) or women who received 3 doses (0%) or at least 2 doses (2.4%) of vaccine compared who received 1 doses 8.5% (28/329). We found evidence of cross-protection for HPV31, HPV 45 after vaccine introduction but it was no significant. Besides, we found slight increases in 4 nonvaccine high-risk HPV types (HPV39, HPV 51, HPV52 and 59). Interestingly, to HPV6/11 infection although the frequency was low in non-vaccinated group (3.3%), after vaccination it was 0.2% to HPV6 and no infection were observed to HPV 11.Finally, although there is a reduction of HPV vaccine type,
the prevalence of other HR-HPV remain high, even a slight increase in the vaccinated population is observed (36.6% non-vaccinated Vs 39.7% vaccinated).

Conclusion

Five years after the introduction of the Colombian HPV vaccination program, a decrease in vaccine-targeted genotypes is evident. Variables such as age, number of doses and application of the vaccine before sexual debut increase the effectiveness of the vaccine. In addition, knowing the actual state of the HPV infection in Colombia allows to evaluate the current vaccination schemes and raises the possibility of inclusion of the nonvalent vaccine in our population.
Quantifying the impact of HPV vaccination of 12 year old girls on cervical disease and cytology performance

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Background / Objectives

Routine school-based HPV immunisation was offered to girls aged 12-13 in Scotland from 2008, with a catch-up programme over 3 years for 14-18 year old girls. To date, we have shown that vaccination of catch-up cohorts is associated with a significant decrease in prevalence of HPV 16/18/31/33/45, in cervical intraepithelial neoplasia of all grades and in cytology performance, particularly the predictive value of low-grade cytology. This reduction is achieved despite probable exposure to HPV before immunisation, particularly of the older catch-up girls.

The first cohort immunised at age 12-13 entered the Scottish Cervical Screening Programme in September 2015 at age 20. A further decline in HPV 16/18/31/33/45 prevalence was observed in these women when compared to the catch-up population. Evidence of herd immunity in the unvaccinated population has also emerged, including for cross-protective types.

To complement the work on viral outcomes we will present data on (1) the level of CIN (2) the performance of cytology in the routinely immunised cohort.

Methods

The Scottish Cervical Call Recall System - the national IT system containing all screening records and also vaccination status - will be interrogated. By June 2017 we will have a minimum of 12 months follow-up on all 31,000 routinely immunised women invited for screening. Linked data on degree of immunisation, on cytological abnormalities and on histological diagnosis following referral for colposcopy will be used to calculate odds ratios by immunisation status for cytological abnormality and histological diagnosis. The cytological abnormalities will be correlated with histological diagnosis to determine performance of cytology as a screening test.

Results

Approximately 31,000 women aged 20 were invited between September 2015 and June 2016. Over 23,000 of these had received 3 doses of vaccine. Initial evidence suggests that high grade disease (CIN2+/HSIL+) is virtually absent routinely.
immunised females. Before immunisation, CIN 2+ was confirmed in 2.84% of women screened at age 20. In the 1995 cohort (immunised at age 12-13), CIN 2+ is present in 0.33% of women. Comprehensive data on disease prevalence and on the performance of cytology as a screening test will be presented.

**Conclusion**

This will be the first population-based demonstration of the clinical effect of bivalent HPV immunisation of girls who are likely to have been HPV-naive at the time of vaccination. The information presented will be of great importance for the design and delivery of screening programmes in all highly vaccinated populations, and for planning cancer prevention strategies in resource-poor countries.
FC 07-07
CELLULAR IMMUNE RESPONSES SIX YEARS FOLLOWING REDUCED-DOSE QUADRIVALENT HPV VACCINE IN ADOLESCENT FIJIAN GIRLS


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Background / Objectives

The World Health Organization has recommended 2-dose HPV vaccine schedule separated by 6 months to girls <15 years old as an alternative to the current 3-dose schedules. However, the long-term protection following reduced-dose schedules is unknown. This study examined long-term immunity by comparing the cellular immune 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 cellular immune responses in terms of IFNγ producing cells and cytokines production (IFNγ, IL-2, TNFa, IL-10 and IL-5) were measured against HPV16 and 18 using the IFNγ-ELISPOT assay and Multiplex Bead Array assay, respectively.

Results

Six years following the last dose of 4vHPV, girls who received 2 doses of 4vHPV (p=0.008) had significantly lower HPV18-specific IFNγ producing cells compared with girls who received 3 doses. Significantly lower cytokine responses (IFNγ: p=0.002;
IL-2: p=0.022; TNFα: p=0.016, IL-10: p=0.018) against HPV18 were also observed in girls who received 2 doses of 4vHPV when compared with girls who received 3 doses. These differences were no longer significant 1 month following a dose of 2vHPV. There was no significant differences in cellular immunity against HPV16 between the 2- and 3-dose groups six years after the last dose of 4vHPV and 1 month after a dose of 2vHPV. Interim analysis for the comparison of cellular responses between 1- and 3-dose groups are ongoing, which showed a similar trend (significant lower responses against HPV18 but not HPV16) as the 2-dose group.

Conclusion

Lower HPV18-specific IFNγ producing cells and cytokines were observed in the 2-dose group when compared with the 3-dose group after 6 years. These data suggest that cellular immunity against HPV18 following reduced-dose schedules may not persist as long as a 3-dose schedule, although the clinical significance is unknown. Despite the lower cellular immune responses, the neutralising antibody responses between the 2- and 3-dose groups were similar as shown previously, although lower antibody responses against HPV18 than HPV16 were observed (Toh et al., Clin. Infect. Dis, 2016). Longer follow-up studies on reduced-dose schedules are needed to determine whether cellular immune responses has a role in the long-term protection against HPV18.

References

The efficacy of vaccine prophylaxis of HPV-associated diseases in the Moscow region

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Background / Objectives

From 2007 to 2015 the absolute number of newly diagnosed cases of cervical cancer (CC) in the Moscow region (MR) increased from 594 to 785; the incidence rate per 100,000 in MR for the last 14 years increased from 7.9 in 2002 to 19.5 in 2015, and mortality rate during the 1st year of follow-up exceeds 12%. The program of vaccine prophylaxis of HPV-associated oncological diseases with quadrivalent vaccine in 12 to 13-year-old girls with a 0-2-6 month regimen has been conducted in MO (18 municipal districts) since 2008.

To conduct the efficacy analysis of the vaccine prophylaxis program.

Methods

The incidence rate of HPV-associated diseases in girls and women in 2009 to 2015 and the vaccination safety with the use of the specially designed register were studied.

Results

20,000 female adolescents were vaccinated during the 9-year program. The decrease in the incidence rate of anogenital condylomas (AC) was registered during the study period: the general incidence rate decreased from 127.2 to 24.7, the incidence rate per 100,000 children – from 63.3 to 11.9, and the incidence rate per 100,000 girls who reside in districts covered with vaccine prophylaxis - from 14.2 to 6.1 per 100,000; the decrease of the incidence rate of AC for the study period in the whole female population from 56.7 to 20.2 (per 100,000) was also reported. A positive trend in the decrease of CC case detection in the 15-24 age group from 0.3 to 0.1 (the rate of the detected CC cases among women of all ages) was registered in districts covered by vaccination; no CC cases in young women were registered. According to the register data, no serious adverse events were reported for the vaccination period; some vaccinated patients became pregnant and delivered healthy children.

Conclusion

The results of the vaccine prophylaxis program of HPV-associated diseases conducted in MR have demonstrated the safety and efficacy of vaccine prophylaxis
using a quadrivalent vaccine in decreasing the incidence rates of anogenital condylomas in girls and cervical cancer in young women.
Hazard of complex regional pain syndrome (CRPS) following HPV vaccination among adolescents in the United States

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Background / Objectives

We estimated the association between adolescent vaccination and incident complex regional pain syndrome (CRPS) in adolescent girls in the U.S.

Methods

We used insurance claims to identify claims for CRPS and adolescent vaccination, including HPV, tetanus-diphtheria-acellular pertussis (Tdap) and meningococcal conjugate vaccine (MenACWY), using diagnosis and procedure codes. We identified 11-year-old girls between 2006-2014 without a prior history of adolescent vaccination or CRPS, and followed them from the 11th birthday until CRPS diagnosis, loss to follow-up, or December 31, 2014. Time-dependent Cox models estimated hazard ratios (HRs) comparing CRPS hazard following recent vaccination (≤30 days) to CRPS hazard following prior (>30 days) or no vaccination. HRs were adjusted for history of physical trauma in the year preceding follow-up or an instance of trauma during follow-up. We then estimated time-dependent HRs for CRPS following recent co-administration of common vaccine combinations. Finally, we identified common health diagnoses received by girls in the sample over follow-up, and estimated time-dependent HRs for CRPS comparing girls with each diagnosis to girls without.

Results

We identified 563 CRPS cases among 1,232,572 girls (incidence rate: 20.6/100,000 person-years). CRPS hazard was not significantly elevated following recent HPV (adjusted HR [aHR]: 1.41, 95% CI: 0.83, 2.40), Tdap (aHR: 1.20, 95% CI: 0.64, 2.59), or MenACWY (aHR: 1.21, 95% CI: 0.57, 2.55) vaccination. Comparing recent or non-recent vaccination to no vaccination, Tdap (HR: 1.59, 95% CI: 1.34, 1.89) and MenACWY (HR: 1.70, 95% CI: 1.43, 2.02) vaccination were associated with CRPS in crude analysis, but were not associated after adjusting for trauma (Tdap - aHR:1.09, 95% CI: 0.91, 1.29; MenACWY - aHR: 1.19, 95% CI: 1.00, 1.42). CRPS hazard comparing concomitant HPV vaccination to HPV vaccination alone was not significantly elevated (aHR: 2.30, 95% CI: 0.80, 6.56). Girls with lower limb injuries had the greatest CRPS hazard compared to girls without (HR: 12.4, 95% CI: 10.4, 14.7). Girls with anxiety disorders had a threefold hazard of CRPS compared to girls without (HR: 3.12, 95% CI: 2.41, 4.04). Common pediatric illnesses (e.g. asthma,
respiratory infections, allergies) were positively associated with CRPS in bivariate analyses.

**Conclusion**

Adolescent CRPS is rare in the U.S., and adolescent vaccination was not significantly associated with CRPS hazard. Crude HRs for CRPS following vaccination were confounded by physical trauma, a known CRPS risk factor. Future research should consider health-related risk factors for CRPS in adolescents, particularly injuries, and inflammatory and mental illnesses.
SYSTEMATIC CAUSALITY ASSESSMENT OF ADVERSE EVENTS FOLLOWING HPV VACCINATION IN ITALY

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Background / Objectives

WHO recommends that serious adverse events following immunization (AEFIs) should be monitored and evaluated using a standardized algorithm for causality assessment. “Per protocol”, after a comprehensive analysis of the event and the concomitant factors, the relationship between the vaccine and the AEFI could be classified as “consistent”, “not consistent”, “undetermined” or “unclassifiable” (1). Despite WHO recommendations, the AEFI causality assessment manual is, in the clinical practice, rarely adopted. In Italy, AEFIs, spontaneously reported from physicians, healthcare workers or patients to the National Drug Authority (AIFA) routine surveillance system, are classified only by temporal criteria. Therefore, the AEFIs report, published yearly by the AIFA, lacks information about the strength of correlation between events and vaccines (2). In this work, we aimed at evaluating the systematic use of Causality Assessment algorithm of AEFIs following HPV vaccination in Italy.

Methods

In the Apulia region of Italy (about 4,000,000 inhabitants), from 2008 to 2014, 438,294 HPV vaccine doses were administered to females aged 12, 18 and 25 years. We selected severe AEFIs following HPV vaccination reported between 2008 and 2014 to the AIFA routine surveillance system. We applied the WHO causality assessment criteria; for AEFIs requiring hospitalization, we repeated the assessment obtaining additional information from individual medical records.

Results

Of 14 severe AEFIs following HPV vaccination (reporting rate: 3.2 x100,000 doses), 8 (57.1%) led to hospital admission. After causality assessment, 7 AEFIs were classified as consistent, 3 undetermined, 2 not consistent, 2 unclassifiable. Among hospitalized cases, 4 AEFIs were classified as consistent, 2 as not consistent, 1 as undetermined and 1 as unclassifiable; adding information from medical records, we obtained similar outcomes with the exception of the “undetermined” AEFI that changed in “not consistent”.

Conclusion
Severe AEFIs following HPV vaccination are rare. The systematic use of the causality assessment algorithm showed that only half of them could be related to vaccination. Information from the AIFA routine surveillance system, in the absence of a causative analysis, provides a distorted picture of the HPV vaccine safety and should be interpreted with caution.

References

1. WHO. Causality Assessment of an Adverse Event Following Immunization (AEFI), User manual for the revised WHO classification. WHO/HIS/EMP/QSS. March 2013

DRAW UP A PROTOCOL FOR THE USE OF VAGINAL SELF COLLECTIONS IN ‘NON RESPONDER’ WOMEN IN TUSCANY HPV PRIMARY SCREENING PROGRAM.

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Background / Objectives

A key feature of a self-collected HPV testing strategy is the move of the primary screening activities from the clinic to the community with the efforts to increase the affordability and availability of HPV DNA tests. Within the self sampling project, ongoing in ISPO, Florence, women aged 35–64 years, residents in Florence, who had been invited by the screening programme, in the past screening round and had failed to respond were eligible for the study. A random sample of 5200 eligible women was randomly assigned to one of the following arms: control arms with a standard invitation letter to perform HPV test at the clinic on a pre-fixed date; two intervention arms: a group was directly sent the “home based” dry self-sampler device (nylon FLOQSwab® Copan, Italy) another group was directly sent the “home based” self-sampler device (nylon FLOQSwab® Copan, Italy) and 1 ml of preservation and transport solutions (MSwab® Copan, Italy). As a prerequisite for carrying out the study, we have investigated sensitivity and reproducibility of HPV test, carried out on Cobas®4800 (ROCHE®) in HPV16 plasmid samples obtained by swirling the FLOQSwab® in ThinPrep® (TP, HOLOGIC®) and in MSwab®+TP®.

Methods

Starting from HC2® (Hybrid Capture®2, QIAGEN®) high risk calibrator (plasmid 100 HPV16 copies/μl) we prepared a series of dilutions in TP® and MSwab®+TP® (rate 1:4). For each test, calibrator was absorbed on FLOQSwab® and then swirled in assay preservation solutions. For each sample clinical sensitivity (5000 HPV copies/reaction) and LOD (Limit of Detection) were evaluated in 20 replicates comparing the results with Roche LOD (600 copies HPV16/ml). FLOQSwab® adsorption was taken in account in order to guarantee the actual copies number.

Results

All 12500 HPV16 copies/ml (5000 HPV16 copies/reaction) replicates in MSwab® +TP® and TP® are positive. Only 65% of 600 copies of HPV16/ml replicates in TP® and 60% in MSwab®+TP® result positive. It was found that LOD is 1200 copies of HPV16/ml, since all replicates in MSwab®+TP® and 95% of those in TP® are positive. FLOQSwab® adsorption was about 230 μl.

Conclusion
FLOQSwab® LOD for HPV16 is 1200 HPV16 copies/ml. Compared to LOD provided by Roche, results show that FLOQSwab®, retained part of the viral load, shows a higher LOD. However near to the clinical sensitivity limit, FLOQSwab®’s performance was as expected. Against this different FLOQSwab® performance, the MSwab® analysis buffer has been shown to have good performance, both for much lower and at the limit of clinical sensitivity viral loads. From the results reported, it appears that MSwab® has a slightly higher analytical sensitivity than TP®, which could however result in a lower clinical sensitivity.
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Background / Objectives
The Capital Region of Denmark is currently implementing HPV self-sampling to screening non-attenders as a new offer in the organized screening program. From the pilot implementation study, offering 24,000 non-attenders an Evalyn self-sampling brush (Rovers, Oss, Netherlands), we have previously described that only 0.3% of the returned brushes returned an invalid result upon HPV testing using the BD Onclarity HPV assay. However, temperatures differences across a calendar year, prolonged storage after sampling or during transport in the mail from the woman to the lab could potentially affect the analytical stability of the dry, self-taken samples. To strengthen the evidence for use of self-sampling, we investigated the analytical stability of the Evalyn dry brush for HPV testing as a function of time and ambient temperatures.

Methods
To simulate self-taken samples, we used residual cervical swabs (Copan Universal Transport Media, SSI, Copenhagen) from Danish women undergoing routine diagnostic HPV testing at Copenhagen University Hospital. Brushes were dipped in residual media, left to dry and then stored at three different temperatures (room temperature, 4°C and 30°C) prior to being analyzed using the clinically validated BD Onclarity HPV assay at four different storage time points, T=0 (baseline), 2, 4, and 8 weeks. Analytical quality of the samples was assessed using the Ct value on the BD Onclarity HPV test internal control Human Beta Globin Control (HBB). Up to four brushes were derived from each swab sample, allowing for longitudinally comparison of different study points. After storage and prior to Onclarity testing, the brush heads were removed and rinsed in 3 ml BD CBD medium. 1.0 ml was used for HPV testing in concordance with manufactures specifications.

Results
The mean Ct value of the HBB outcome per sample was compared. No statistical difference was observed in HBB Ct values between baseline and T=2 weeks, 4, and 8 weeks regardless of storage temperature (4°C; p=0.951, Room temperature; p=0.763, 30°C; p=0.203). All samples were reproducible with respect to HPV result and the Ct values of the individual HPV genotype groups were stable throughout the time points.
Conclusion

This data conclusively shows that dry brushes used for HPV self-sampling are analytically stable with respect to human and HPV DNA up to 8 weeks after the actual sampling, as well as over temperature conditions ranging from 4C to 30C. This provides important data for implementation of HPV self-sampling worldwide under different temperature and environment conditions.
INCREASING SCREENING ATTENDANCE AMONG LONG-TERM SCREENING NON-ATTENDERS: RANDOMIZED HEALTHCARE POLICY


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Background / Objectives

The organized cervical screening program of Stockholm, Sweden reaches a 10-year population coverage of 96%, with the remaining 4% of the population constituting a high risk group for cervical cancer. The organized coordination and quality assurance allows for pilot implementation of novel screening strategies designed to increase coverage among long-term non-attenders. We performed a randomized health services study within the real-life organized screening program.

Methods

A comparison of the population registry with the regional screening registry identified that 16,437 out of the 413,487 resident women between 23 and 60 years of age had not taken a cervical screening test in at least 10 years, despite annual renewed invitations. Among these long-term non-attenders, 8000 women were randomized to either a) ordering a self-sampling kit using an open source e-Health application b) mailing a HPV self-sampling kit directly to the woman c) an invitation to call the coordinating midwife of the screening program with questions and concerns regarding screening; and d) standard annual renewed invitation letter (routine practice). HPV positive women were referred directly to colposcopy. Participation rates by study arm and outcome of screening tests were identified by registry linkages.

Results

Overall participation, defined as returning a self-sampling kit (or other screening participation) by arm was as follows: a) 10.7%; b) 18.7%; c) 1.9%; and d) 1.7%. The relative risk of participating in study arm a was 6.3 (4.4-8.9), 11.0 (7.8-15.5) in arm b, and 1.1 (0.7-1.7) in arm c, compared to routine practice (repeat renewed invitation with new appointment) in study arm d. HPV prevalence among women who returned
kits in study arms a and b was 12.2%. In total, 63 women were referred to colposcopy, out of which 44 women attended.

Conclusion

Offering self-sampling increased attendance, even among women who had not responded to more than 10 invitations with appointments in an organized program. Attendance was higher when kits were sent directly but offering women to order a kit did increase attendance at lower costs.
THE CHOICE TRIAL: A RANDOMIZED, CONTROLLED EFFECTIVENESS TRIAL OF HPV SELF-SAMPLING FOR NON-PARTICIPANTS IN AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

Offering cervico-vaginal self-sampling for high-risk human papillomavirus testing (hrHPV self-sampling) to non-participants in a cervical cancer screening program may increase uptake depending on the delivery mode of the self-sampling offer. We compared the effectiveness of different approaches for delivering a self-sampling offer to non-participants in an organized program in terms of screening up-take, and analyzed the proportion of self-samplers that received appropriate follow-up.

Methods

The study included 9,791 women aged 30-64 from the Central Denmark Region who have not participated in cervical cancer screening despite invitation and one reminder. They were randomized 1:1:1 to either: 1) direct mailing of a HPV self-sampling kit (directly mailed group); 2) mailing of an offer to order a self-sampling kit by either e-mail, text message, phone, or webpage (opt-in group); or 3) mailing a second reminder to contact a general practitioner (GP) for usual care, viz. cytology (control group). Women offered self-sampling were informed that they could also receive usual care if wanted. The self-sampling kit comprised a brush device (Evelyn Brush) for hrHPV testing using Roche Cobas® 4800. Performing an intention-to-treat analysis, we estimated the up-take 180 days post intervention, including self-samples taken at home and cytologies taken at a GP. Self-samplers’ compliance with GP follow-up was measured 90 days after a hrHPV-positive test result.

Results

The up-take was significantly higher in the directly mailed group (37.0%) than in the opt-in group (29.9%) (absolute participation difference (PD): 7.1%, 95% CI: 3.1-11.1%) and the control group (24.1%) (PD: 13.0%, 95% CI: 8.8-17.0%). Of 118 hrHPV-positive self-samplers, 91.0% (107) attended follow-up. Self-samplers were
significantly less likely than controls to have been screened in the previous screening round (30.8% vs. 12.8%, RR: 0.42, 95% CI: 0.34-0.51). We estimated an overall participation rate of 71% in the directly mailed group, 68% in the opt-in group, and 65% in the control group. The direct mailing strategy increased the absolute overall participation among invited women by 6.0%, (95% CI: 5.8-6.2%).

Conclusion

Direct mailing of self-sampling kits to non-participants proved to be the most effective strategy for increasing screening participation. Using timely opt-in procedures yielded an only limited participation gain compared with a second reminder to attend regular screening. Most hrHPV-positive women had appropriate follow-up. Implementing self-sampling in the Danish screening program may increase overall up-take and help recruit under-screened women, thereby increasing the program's effectiveness.
HOME-BASED HPV SELF-SAMPLING TO INCREASE CERVICAL CANCER SCREENING PARTICIPATION: A PRAGMATIC RANDOMIZED TRIAL IN A U.S. HEALTHCARE DELIVERY SYSTEM

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Background / Objectives

Women who delay Pap screening are at increased risk for cervical cancer. In countries with organized screening programs, population-based randomized controlled trials (RCTs) have shown that mailing HPV self-sampling kits to underscreened women increases participation. Our objective was to evaluate the effectiveness of this approach in a U.S. healthcare system.

Methods

We conducted a pragmatic RCT within Kaiser Permanente Washington (an integrated healthcare system) to compare 2 programmatic approaches for increasing screening among women aged 30-64 years who were overdue (≥3.4 years since last Pap). The control arm included usual care (annual patient reminders and ad hoc outreach by clinics). The intervention arm included usual care plus a mailed self-sampling kit for HPV testing by Roche Cobs assay. Women and their healthcare providers were notified of the home HPV results and providers were responsible for encouraging women to receive appropriate follow-up: diagnostic colposcopy if HPV16/18+ and additional in-clinic screening (Pap or co-test) if unsatisfactory or positive for non-HPV16/18 types. Unlike similar trials in other countries, HPV-negative women were still recommended to receive in-clinic screening because home HPV testing is not an approved screening strategy in the U.S. Screening uptake was defined as any of the following within 6 months of randomization: 1) in-clinic screening; 2) returning a kit that was HPV-negative or HPV16/18+; or 3) returning a kit that was unsatisfactory or positive for non-HPV16/18 types, followed by in-clinic screening.

Results

From 2014-2016, we randomized 16,242 women (8116 control; 8126 intervention) with a median age of 51 years. Screening uptake was higher in the intervention than
control arm (28.3% vs. 19.5%; relative risk=1.45, 95%CI:1.37-1.54). Within the intervention arm, 13.1% of women returned a kit and 15.4% attended in-clinic screening without returning a kit. 11.5% of self-sampling kits were HPV+ (3.1% HPV16/18+ and 8.4% positive for non-HPV16/18 types only).

Conclusion

Mailing HPV self-sampling kits to underscreened women increased screening uptake compared to usual care alone. Screening uptake in our intervention arm was comparable to pooled uptake from a meta-analysis of non-U.S. trials (28.3% vs. 23.6%).[1] Our results suggest that approximately half of women who choose to get screened after receiving a mailed home HPV kit will choose in-clinic screening over self-sampling in a hybrid screening approach. Additional findings from this study will describe clinical outcomes, demographic and health predictors of screening uptake, and self-sampling experiences and attitudes.

References


ClinicalTrials.gov, NCT02005510
FC 08-06

COMPARATIVE EVALUATION OF TWO CERVICOVAGINAL SELF-COLLECTION METHODS TO DETECT THE PRESENCE OF CLINICALLY SIGNIFICANT HUMAN PAPILLOMAVIRUS INFECTION.


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Background / Objectives

PCR based high-risk human papillomavirus (hrHPV) testing on self- and physician-collected specimens have shown similar sensitivity to detect a high-grade cervical lesion (CIN 2+). However, limited evidence is available on the performance and acceptability of different sampling devices. We compared two self-collection methods to detect clinically significant hrHPV infection in Norway.

Methods

310 women referred to the treatment for histologically verified CIN 2+ completed self-collection using a dry brush (Evalyn Brush, Rovers, Netherlands) and a dry swab (FLOQSwabs, Coban, Italy), and filled a questionnaire. At the hospital, a physician took an additional cervical specimen (PreservCyt, Hologic, USA). Cytology specimens were blindly analysed by one cytotechnician and one pathologist. Self-specimens and a physician-specimen (reference) were analysed for the presence of 14 hrHPV DNA using three commercially available HPV assays; Anyplex (Seegene, South Korea), Cobas (Roche, USA) and Xpert HPV (Cepheid, USA). Agreement between self- and physician-specimen was assessed with kappa (κ) statistics with bootstrapped 95% confidence intervals (CIs). Absolute and relative sensitivities with 95% confidence intervals were computed using a matched-pair design.

Results

Analyses included 251 women with matched triplets. Overall hrHPV positivity rate was 89% in the reference specimen using Anyplex, and 86% using Cobas and Xpert HPV.

Overall agreement for hrHPV positivity between self- and physician-specimens was highest at 94% using Anyplex on Evalyn (κ = 0.68, 95% CI: 0.53-0.83), and lowest at 82% using Xpert HPV on FLOQSwabs (κ = 0.47, 95% CI: 0.35-0.59). Anyplex detected 95% of the CIN2+ lesions. Corresponding sensitivities for Cobas and Xpert HPV were 93% and 94%. The ability of any hrHPV test to detect CIN2+ from a brush-
specimen was similar to the reference, whereas significantly lower sensitivities were demonstrated using a swab. Both devices were well accepted, but women considered a brush easier, less painful and less uncomfortable than a swab. Generally, we did not observe any differences on perceptions on self-collection by sociodemographic status.

**Conclusion**

We observed significant device effects in detection of the hrHPV DNA and CIN2+ using three validated HPV assays. There were also differences on the acceptability of the sampling devices. If self-collection is considered as an alternative to provider-collection in national screening programmes, both hrHPV assays and sampling devices have to be validated.
HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA IN HUMAN PAPILLOMAVIRUS SELF-SAMPLING OF SCREENING NON-ATTENDERS VERSUS ROUTINELY SCREENED WOMAN

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Background / Objectives

Self-sampling for Human Papillomavirus (HPV) offered to women who do not participate in organized cervical cancer screening is an increasingly popular method to increase screening coverage. The rationale behind it is that under-screened women harbour a high proportion of undetected precancerous lesions since ~50% of disease is routinely detected in underscreened women. In 2014 the “Copenhagen Self-sampling initiative” (CSI) offered HPV self-sampling to screening non-attenders in the Capital Region of Denmark. We compared the ≥CIN2-detection rate between screening non-attenders, who participated in self-sampling, and women attending routine screening (The HORIZON cohort).

Methods

23,632 women who were qualified as screening non-attenders in the Capital Region were offered HPV-based self-sampling using an Evalyn brush (Rovers, Oss the Netherlands). 4824 (20.6%) women returned a self-sample brush, and HR-HPV-positive women were referred for cytology and HPV-testing as follow-up. The entire cohort and a reference cohort (3347 routinely screened women from the HORIZON cohort), were followed for histopathology-confirmed ≥CIN2. Odds ratio and positive predictive value of ≥CIN2-detections between the two populations were estimated

Results
Women participating in self-sampling had the same ≥CIN2-detection rate as routinely screened women (OR = 1.03; 95% CI: 0.75-1.40). The positive predictive value of ≥CIN2 detections was, however, higher in screening non-attenders than routinely screened women (36.5% vs. 25.6%, respectively). Among all detected ≥CIN2, women were slightly more likely to have ≥CIN3 detected if they were CSI-attenders (78.6% of all ≥CIN2 diagnoses were ≥CIN3), than if they were routinely screened (HORIZON population) (72.1%). However, in total, 18 women were diagnosed with cervical cancer in the screening non-attenders population, versus only one in the reference population.

**Conclusion**

Self-sampling to non-attenders had similar detection rates for ≥CIN2 as routine cytology-based screening, reinforcing the importance of self-sampling to screening non-attenders in organized cervical cancer screening. The proportion of high-grade CIN lesions among all biopsies was high, demonstrating the efficiency of the approach. The major finding was a large increase in cancer detection in the self-sampled group, which underlines the importance of reaching underserved women to reduce morbidity and mortality from cervical cancer.
HPV test using self-sampling device is useful and effective in non-attendees of cervical cancer screening in Japan: In municipal population based screening in Izumo city

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Background / Objectives

In Japan, incidence and mortality of cervical cancer is increasing especially in young women in their late 20s and early 30s. One of the considerable factors is the low coverage of screening which is only 32.7% according to the National Livelihood Survey 2013. We tried to use self-sampling HPV test to approach non-attendees in Izumo city, which has implemented HPV co-testing for cervical cancer screening since 2007. 2,120 out of 12,546 women who were between 25-45 years old and did not attend municipal cervical cancer screening from 2010 to 2014 conducted HPV self-sampling test in 2015. At our presentation in EUROGIN 2016, the acceptability of self-sampling HPV test was very good. We want to make sure if HPV test using a self-sampling device is really useful and effective for non-attendees. So we investigate how many women who conducted self-sampling HPV test went to the medical facility and received the cytology, especially as a municipal cervical cancer screening after they got the result of HPV test.

Methods

The candidates of this study were 2,120 women living in Izumo city, who conducted HPV self-sampling test in 2015. We followed up on their results of cytology and HPV test using physician collected samples. We sent a letter and a questionnaire to those who were HPV positive and did not receive municipal screening to find out if they receive screening in any other setting or not.

Results

In 2,120 attendees of self-sampling HPV test, 152(7.2%) of women were screen-positive and 1,968(92.8%) women were screen-negative. 123(89.2%) of screen-positive women received cervical cancer screening. 111 out of 123 receive municipal screening (cytology and physician-collect HPV test) in Izumo city, and 40 of them had a detailed examination. The detection rate of CIN2+ in those who conducted self-sampling HPV test is 10.8%, and 18 times as many as the rate of CIN2+ in general screening run by Japan Cancer Society, which covers one third of population based cervical cancer screening in Japan. 239(12.1%) of screen-negative women receive municipal screening. 362 women (17.1% of the total 2,120) who conducted self-sampling HPT test went to the medical facility to undergo cytology as municipal screening. It is also 2.9% of total non-attendees in 5 years.
Conclusion

We believe that using self-sampling HPV test is a very useful and effective way to approach non-attendees of municipal cervical cancer screening. In Japan, there are many areas including small islands with few or no available facilities. Not only for non-attendees but also for women in low resource area in Japan, self-sampling HPV test has a possibility to improve the health and wellness of Japanese women.
PRIMARY HPV SCREENING USING THE COBAS® HPV TEST ON SELF-COLLECTED DRY CERVICOVAGINAL SAMPLES FROM UNDERSERVED GREEK WOMEN. PRELIMINARY RESULTS OF THE GRECOSELF STUDY


Background / Objectives

To assess the performance of HPV-based cervical cancer screening in underserved women in Greece using the cobas® HPV Test on self-collected cervicovaginal samples, in comparison to historical real-life results of cytology-based screening. Secondary objective is to assess the acceptability of the Roche® self-collection device for cervicovaginal specimens.

Methods

Women 25-60 years old who do or do not attend cervical cancer screening and reside in rural areas of Greece are being recruited for the study. Approximately 12,700 women will be enrolled over 30 months starting May 2016. Women are contacted by midwives, forming a nationwide midwifery network set for the study purposes, via public announcement at their place of residence, and are provided, after giving their written informed consent, with a self-sampling kit (Roche®) (cotton swab and dry vial) along with the necessary instructions. Each woman collects the specimen and fills in a questionnaire specifically designed to assess cervical cancer screening participation and outcome history during the last 10 years, and the acceptance of the self-sampling procedure. Samples are tested using the cobas® HPV Test, Roche®, which detects HPVs 16 and 18 separately, and HPVs 31,33,35,39,45,51,52,56,58,59,66 and 68 [other high-risk (OHR)] as a pooled result. Women found positive for HPV are being referred for colposcopy. In case of abnormal colposcopic impression a biopsy is taken. If the histology report is within normal limits the woman is referred to routine screening, if there is Cervical
Intraepithelial Neoplasia (CIN) grade 1 or 2 or worse (CIN2+) she is referred to follow up or appropriate treatment respectively.

Results

Between May 2016 and March 2017 6,818 samples were collected, 6,156 were tested, of which 433 (7.0%) were HPV positive, 228 colposcopies were performed and CIN grade 1, 2 and 3 was detected in 17, 12, and 10 cases respectively. HPV16 positive cases were found in 14.2% and 63.6% among the low-grade (CIN1) and high-grade (CIN2+) lesions respectively. Moreover there had been a case of vaginal intraepithelial neoplasia (VaIN – OHR positive) and a case of cervical adenocarcinoma (HPV16 positive). The prevalence of high-grade disease or cancer among HPV positive women examined was 10.5% and among tested women overall 0.4%, about half than expected, since only about half of the HPV positive women have been examined colposcopically so far.

Conclusion

The preliminary report of the GRECOSELF study shows that HPV testing with individual HPV 16/18 genotyping on self-collected cervicovaginal samples is a feasible and effective cervical cancer prevention method for Greek women residing in rural distant areas.
THREE-YEAR EFFICACY OF THE QUADRIVALENT HPV VACCINE IN A COHORT OF HIV-POSITIVE WOMEN

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Background / Objectives

To assess the efficacy of the qHPV vaccine at 3 years in our cohort of HIV-positive women.

Methods

HIV-positive females participating in a multi-centre study of the qHPV vaccine were administered three doses at 0, 2 and 6 months. Demographic and clinical data were collected as well as samples for cervical cytology, classified by Bethesda criteria, and HPV DNA tested by Linear array assay. Persistent qHPV, genital warts and CIN2+ were assessed. Persistent cases of qHPV were defined as new HPV 6/11/16/18 that remained present in samples from ≥2 consecutive visits or as qHPV present in the last sample.

Two-year data was compared to findings in HIV-negative women to provide context to our results. To improve comparability of the cohorts, HIV-positive women with history of genital warts, cervical disease and cervical procedure were excluded as these were exclusion criteria among the HIV-negative women. A composite endpoint comprised of the three previous endpoints was utilized.

Results

278 females were eligible for the intention-to-treat (ITT) population (≥1 dose of vaccine, ≥1 follow-up visit). At first vaccination, median age was 39 years (IQR: 34-45), median CD4 count was 499/mm3 (IQR: 380-654), median CD4 nadir was 230/mm3 (IQR: 120-337) and 72% had a suppressed HIV viral load (<50 copies/mL). Median follow-up was 3.2 years. In the per-protocol efficacy (PPE) population (3
doses of vaccine within 1 year, ≥1 follow-up beyond month 7, naive to the relevant qHPV type), persistent qHPV = 0.9 per 100 person-years (95% CI: 0.4-1.8), genital warts =1.0 per 100 person-years (95% CI: 0.4-2.0), CIN2+ = 0 per 100 person-years.

Within 2-year follow-up of HIV-positive women, composite endpoint incidences in the PPE, NRT and ITT groups were 1.4, 2.3 and 3.7 per 100 person-years, respectively. Among HIV-negative vaccinated women, incidences were 0.1, 0.5 and 2.7 per 100 person-years. Among HIV-negative unvaccinated women, incidences were 1.5, 2.0, 3.9 per 100 person-years.

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† Muñoz et al., 2009.

Conclusion

This study demonstrates overall low rates of vaccine failure with low rates of infection and/or disease. However, the rates of qHPV-related disease were notably higher than rates in vaccinated HIV-negative women based on published studies and equivalent to rates in unvaccinated HIV-negative women.

References

THREE-YEAR EFFICACY OF THE QUADRIVALENT HPV VACCINE IN A COHORT OF HIV-POSITIVE WOMEN

E. McClymont 1, M. Lee 1, J. Raboud 2, S. Blitz 2, F. Coutlée 3, S. Walmsley 4, N. Lipsky 5, D. Money 1

1University of British Columbia (Canada), 2University Health Network (Canada), 3University of Montreal (Canada), 4University of Toronto (Canada), 5Women’s Health Research Institute (Canada)

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HIV-positive females participating in a multi-centre study of the qHPV vaccine were administered three doses at 0, 2 and 6 months. Demographic and clinical data were collected as well as samples for cervical cytology, classified by Bethesda criteria, and HPV DNA tested by Linear array assay. Persistent qHPV, genital warts and CIN2+ were assessed. Persistent cases of qHPV were defined as new HPV 6/11/16/18 that remained present in samples from ≥2 consecutive visits or as qHPV present in the last sample.

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Results

278 females were eligible for the intention-to-treat (ITT) population (≥1 dose of vaccine, ≥1 follow-up visit). At first vaccination, median age was 39 years (IQR: 34-45), median CD4 count was 499/mm3 (IQR: 380-684), median CD4 nadir was 230/mm3 (IQR: 120-337) and 72% had a suppressed HIV viral load (<50 copies/mL). Median follow-up was 3.2 years. In the per-protocol efficacy (PPE) population (3 doses of vaccine within 1 year, ≥1 follow-up beyond month 7, naive to the relevant qHPV type), persistent qHPV = 0.9 per 100 person-years (95% CI: 0.4-1.8), genital warts = 1.0 per 100 person-years (95% CI: 0.4-2.0), CIN2+ = 0 per 100 person-years.

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person-years. Among HIV-negative unvaccinated women\(^1\), incidences were 1.5, 2.0, 3.9 per 100 person-years.

**Conclusion**

This study demonstrates overall low rates of vaccine failure with low rates of infection and/or disease. However, the rates of qHPV-related disease were notably higher than rates in vaccinated HIV-negative women based on published studies and similar to rates in unvaccinated HIV-negative women.

**References**

IMPACT OF BASELINE COVARIATES ON THE IMMUNOGENICITY OF 9-VALENT HPV VACCINE IN MEN AGE 16-26 YEARS

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Background / Objectives

A 9-valent HPV (6/11/16/31/33/45/52/58) (9vHPV) vaccine was developed to provide protection against the HPV types already covered by the quadrivalent HPV (6/11/16/18) (qHPV) vaccine and the five HPV types most commonly associated with cervical cancer worldwide after HPV 16/18. Antibody response to prophylactic HPV vaccines is the basis for their effectiveness in preventing infection and disease related to vaccine HPV types. Here, we present the combined results of an analysis of 9vHPV vaccine immunogenicity in men age 16-26 years in clinical trials.

Methods

Immunogenicity analyses of two clinical trials of the 9vHPV vaccine (protocols 003 [NCT01651949] and 020 [NCT02114385]) were conducted by competitive Luminex immunoassay in males age 16-26 years in a per-protocol immunogenicity population (PPI) consisting of subjects seronegative at Day 1 for the tested HPV type. Immunogenicity was summarized in populations defined by age at vaccination (≤21 or >21 years of age), sexual orientation (heterosexual men [HM], men having sex with men [MSM]), race, and region of residence. Immunogenicity in a historic clinical trial of the qHPV vaccine in men age 16-26 years was also considered.

Results

Of the randomized subjects, 1665 (99.8%) received at least one injection of 9vHPV vaccine. More than 99% 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, geometric mean titers (GMT) for all nine HPV types were robust across age, sexual orientation, race, or region of residence. The magnitude of anti-HPV response to 9vHPV vaccine tended to decrease with increase in enrollment age. It was lower in MSM compared with HM with GMT ratios (MSM/HM) at 4 weeks post-Dose 3 ranging from 0.58 to 0.74, depending on the HPV type in the 9vHPV vaccine trials. GMTs were also lower in MSM versus HM in the historic qHPV vaccine trial, with GMT ratios ranging from 0.49 to 0.66. GMTs remained lower in MSM compared with HM after adjusting for age and region of residence in both vaccine clinical programs.

Conclusion

The 9vHPV vaccine was strongly immunogenic, as shown by high seroconversion rates (>99%) for all vaccine HPV types and robust HPV antibody responses.
regardless of race, geographic region, age, or sexual orientation. The lower immunogenicity in MSM compared with HM was not due to differences in baseline characteristics of age or region of residence and does not appear to have a meaningful clinical significance, as the qHPV vaccine was previously shown to be highly efficacious to prevent HPV infection and disease in MSM.
PREVENTING HPV RELATED DISEASES: AN HEALTH TECHNOLOGY ASSESSMENT OF THE NINE-VALENT VACCINE IN ITALY

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Background / Objectives

The human papillomavirus (HPV) is the most common viral infection of the reproductive system and the second viral agent responsible for cancer. About 5% of cancers can be attributed to HPV; the most common is cervical cancer, but also other cancers are due to HPV. Five-years survival rates are very low except for cervical cancer thanks to the screening. Genotypes 16 and 18 are responsible of around 70% of cervical cancers, but considering also 45, 31, 33, 52, 58 and 35, the proportion reaches 90%. Most of them are preventable through vaccination. The objective of this report is to assess the impact of the implementation of a universal HPV vaccination campaign with a nine-valent vaccine in Italy using the rigorous methodology of the Health Technology Assessment (HTA).

Methods

A HTA has been developed considering all the available evidence on epidemiological, clinical effectiveness, safety, cost-effectiveness, organizational, social and ethical aspects with a focus on Italian population.

Conclusion

A great amount of evidence is available regarding HPV epidemiology, vaccine efficacy and safety and the economic impact of a national vaccination program, but this is the first attempt to collect them together using HTA. The report highlights that
A nine-valent universal vaccination is cost-effective in reducing the risk of HPV-related cancers and diseases. The introduction of a nine-valent vaccine extends the protection to an increasing number of genotype and, consequently, can avoid a huge amount of neoplastic lesions in different sites and reduce the burden of disease. In fact, around 88% of anal, 70% of vaginal, 50% of penile and 43% of vulvar cancers are due to HPV. The nine-valent vaccine represents an investment for public health as demonstrated by the favorable cost-effectiveness profile under the perspectives of both the National Health Service and the society. This is strictly related to a reduction of healthcare costs to manage HPV-related diseases and to an increasing patients’ quality of life. From an organizational point of view, schools have been identified as one of the most effective settings for immunization campaigns. Additionally, amelioration in the invitation letters, communication strategies and/or activities aimed at strengthening the involvement of health care workers emerged as possible determinants of a higher vaccination coverage and a reduction of access inequality. A universal vaccination with nine-valent vaccine is an ethical choice for the society due to the higher clinical benefit-non-maleficence ratio than available alternatives for both the single vaccinee and the entire society considering also the incremental benefit due to the herd immunity.

References


SAFETY OF HUMAN PAPILLOMA VIRUS 9-VALENT VACCINE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background / Objectives

Vaccination against human papillomavirus (HPV) has been progressively implemented in most developed countries for approximately 10 years. In order to increase the protection of the vaccines, a 9-valent vaccine (HPV9) was developed, which provides protection against nine types of the virus, but is not yet used in prevention programs. Studies evaluating its safety are rare. Thus, in this study we performed a meta-analysis of three clinical trials assessing adverse effects in women randomly vaccinated with HPV9 or tetravalent vaccine (HPV4), with the objective of analyzing whether the HPV9 is as safe as HPV4.

Methods

A systematic review and metaanalysis of the HPV vaccines' safety in women was made. Randomized controlled trials (RCT) published between 2011 and 2016 were identified from searches of PubMed, Embase, Scopus, Web of Science and the SciELO databases.

Results

The studies selected 27,465 women who received one of the two vaccines. Results showed that pain (OR 1.72; 95% CI 1.62-1.82) and erythema (OR 1.29; 95% CI 1.21-1.36) occurred significantly more in HPV9 group. However, there was no significant difference between groups for the following adverse effects: headache (OR 1.07; 95% CI 0.99-1.15), dizziness (OR 1.09; 95% CI 0.93-1.27) and fatigue (OR 1.09; 95% CI 0.91-1.30) and the occurrence of serious events related to vaccination was similarly rare among those vaccinated.

Conclusion

Therefore, our findings demonstrate that HPV9 in female patients is as safe as the tetravalent vaccine.

References


9-VALENT HPV VACCINE EFFICACY AGAINST RELATED DISEASES AND DEFINITIVE THERAPY: COMPARISON TO HISTORIC PLACEBO POPULATION

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Background / Objectives

The 9-valent human papillomavirus (HPV) vaccine protects against the same four HPV types as the quadrivalent HPV vaccine and five additional oncogenic types. The pivotal efficacy study of the 9-valent vaccine was controlled with the quadrivalent vaccine. Since the trial had no placebo group, a direct comparison with an unvaccinated population was not possible. Here, we present efficacy analyses comparing the 9-valent vaccine group with a historic placebo population.

Methods

Three international, randomized, double-blind studies were conducted using the same methodology. In the efficacy study of the 9-valent vaccine (Protocol V503-001; NCT00543543), 7106 and 7109 women received the 9-valent or quadrivalent vaccine, respectively. In the historic efficacy studies of the quadrivalent vaccine (FUTURE I [NCT00092521] and II [NCT00092534]), 8810 and 8812 women received the quadrivalent vaccine or placebo, respectively. Cervical cytologic testing was performed regularly. Tissue samples from biopsy or definitive therapy (loop electrosurgical excision procedure, conization) were assessed for HPV DNA.

Results

Among women negative to 14 HPV types prior to vaccination with the 9-valent vaccine, the incidence of high-grade cervical disease and cervical definitive therapy related to the nine HPV types was reduced by 97.4% (95% CI 91.0, 99.5) and 96.6% (95% CI 90.5, 99.1), respectively. The 9vHPV vaccine did not prevent disease related to HPV types detected at baseline but significantly reduced high-grade cervical disease related to other types.

Conclusion

Effective implementation of the 9-valent vaccine may substantially reduce the burden of cervical disease and related health care costs.
FC 09-06
HIGH VACCINE EFFECTIVENESS AGAINST PERSISTENT HPV INFECTIONS UP TO SIX YEARS POST-VACCINATION WITH THE BIVALENT VACCINE IN A COHORT OF YOUNG DUTCH FEMALES

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Background / Objectives

Monitoring vaccine effectiveness (VE) in large-scale vaccination programs is of great importance for assessing the population impact of immunization. This study aimed to estimate the VE of the bivalent HPV vaccine against 12-month type-specific persistent infection up to six years post-vaccination among young Dutch women.

Methods

In 2009, girls 14-16 years of age, who were eligible for the HPV catch-up vaccination campaign in the Netherlands, were invited for participation in a cohort study. Both vaccinated and unvaccinated girls were included and baseline measurements were performed before vaccination. Yearly, questionnaire data and vaginal self-swabs were obtained. Vaginal self-swab material was analyzed with the SPF10-LIPA system. Persistence was defined as at least two consecutive measurements testing positive for the same HPV type, preceded by a high-risk (hr) -negative measurement at baseline or two type-specific negative measurements during follow-up. Type specific hazard ratios were obtained through survival analysis by using the Prentice Williams-Peterson total-time approach, adjusting for ethnicity, age, and sexual behavior. We calculated VE as (1-hazard ratio)*100%.

Results

In total 1593 women (46% vaccinated, 54% unvaccinated) had an available baseline sample, were unvaccinated or vaccinated completely in accordance to the Dutch schedule (at that time 3 doses at 0, 1 and 6 months) and negative for high-risk (hr) HPV at baseline. High VE was observed against vaccine types HPV16 and HPV18 of 95% (95%CI 66%-99%) and 100% (hazard rates per 100 person years: unvaccinated 2.07 (95% CI 1.12-3.86) and vaccinated 0.00 (95%CI 0.00-0.14)), respectively. We observed significant cross-protection against HPV31 (73%, 95%CI 3%-92%). We estimated a VE of 16% (95%CI -14-38%) against all hrHPV-types combined, and a VE of 51% (95%CI 24-69%) against hrHPV-types included in the nonavalent HPV vaccine (HPV16/18/31/33/45/52/58).
Conclusion

The bivalent vaccine shows high effectiveness against 12-month persistent infections by HPV16 and HPV18 among young Dutch women, vaccinated at age 14-16 years while hrHPV negative at baseline, up to six years post-vaccination. Additionally, we found significant cross-protection against 12-month persistent infections by HPV31.
THE HEALTH ECONOMIC IMPACT OF CROSS PROTECTION DUE TO HPV VACCINE

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Background / Objectives

To provide data on the health and financial impact of cross and direct protection from HPV vaccines with a focus on duration of protection.

Methods

A previously validated HPV health economics model calibrated for France was adapted to model cross protection while varying the duration of effectiveness of cross protection and direct protection for the bivalent and the 9-valent HPV vaccines, respectively. Published reports of both clinical trial data and real world evidence show short term protection due to cross protection, while effectiveness from immunity to HPV type-specific virus like particles (VLP) is expected to provide long term protection.

Results

The results from the model show significant reductions in cancer cases, deaths and costs due to long-term protection by type-specific VLP-based vaccines. We consider outcomes for cervical and anal cancers in both male and female population. The 9-valent HPV vaccine compared to the bivalent HPV vaccine with short term (5 years) cross protection results in 74 (per 100,000) fewer cancer cases; 24 (per 100,000) fewer cancer related deaths; and saves €101,850 (per 100,000) over a 100 year time horizon.

Conclusion

Short lived cross protection results in additional cancer cases, cancer-related deaths and lower economic benefit compared to immunity resulting from HPV type-specific VLP-based vaccines.
DECONSTRUCTING EFFICACY AGAINST HIGH-GRADE DISEASE IRRESPECTIVE OF TYPE OF AS04-HPV-16/18 VACCINE AND HPV-6/11/16/18 VACCINE: A POST-HOC ANALYSIS FROM PHASE III TRIALS

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Background / Objectives

A recent systematic review reports substantial heterogeneity in estimates of efficacy against high-grade cervical diseases irrespective of type for AS04-HPV-16/18 vaccine (AS04-HPV16/18) and HPV-6/11/16/18 vaccine (4vHPV).[1] In the current post-hoc analysis, we further explore the reported differences in efficacy.

Methods

Case counts of Cervical Intraepithelial Neoplasia of grade 2 and 3 (CIN2/3) cases were extracted from the FUTURE I/II study (NCT00092521/NCT00092534, Intention-to-Treat naive cohort) [2] and the PATRICIA study (NCT00122681, Total Vaccinated naive Cohort). Cases were assigned to the following categories based on HPV types found in the lesions:

- Lesions with at least one HPV vaccine type* and without any non-vaccine type**
- Co-infections: Lesions with at least one vaccine type* and at least one non-vaccine type**
- Lesions where no vaccine type* was detected (non-vaccine types** only or no high-risk HPV)

Efficacy against lesions with vaccine types, irrespective of type as well as cross-protective efficacies with both inclusion and exclusion of the co-infection cases are calculated using the case counts (n) and totals (N) as extracted.

Results

<table>
<thead>
<tr>
<th>Efficacy (95% CIs) [n vaccine/n control]</th>
<th>Efficacy against lesions with at least one HPV vaccine type* including co-infections with a non-vaccine type**</th>
<th>Efficacy irrespective of type</th>
<th>Cross-protective efficacy including co-infections of a non-vaccine type** with an HPV vaccine type*</th>
<th>Cross-protective efficacy excluding co-infections of a non-vaccine type** with an HPV vaccine type*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATRICIA CIN2</td>
<td>98.9% (93.9 ; 100)</td>
<td>63.3% (50.4 ; 73.2)</td>
<td>47.5% (27.3 ; 62.3)</td>
<td>15.9% (-20.6 ; 41.6)</td>
</tr>
</tbody>
</table>
### Conclusion

No head-to-head efficacy trials that compare different HPV vaccines have been conducted and methodological differences between trials cannot be excluded. However, our post-hoc analysis suggests that efficacy against CIN2 and CIN3 irrespective of type is largely influenced by lesions in which no vaccine type was found, resulting in different estimates for AS04-HPV16/18 and 4vHPV.

*Vaccine types in AS04-HPV16/18: HPV-16/18, 4vHPV: HPV-6/11/16/18


### References


**Funding:** GlaxoSmithKline SA

Conflicts of interest: MR, VB, NK and FS are employees of the GSK group of companies. MR, VB and FS also report shares from the GSK group of companies.
FC 09-09
BIVALENT VACCINE EFFECTIVENESS AGAINST TYPE-SPECIFIC HPV DNA POSITIVITY: EVIDENCE FOR CROSS-PROTECTION AGAINST ONCOGENIC TYPES

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Background / Objectives

To calculate the bivalent vaccine effectiveness (VE) against high-risk HPV DNA positivity, using cross-sectional data from the Netherlands up to six years post vaccination.

Methods

We included all vaccine-eligible women from the PASSYON study, a biennial cross-sectional survey among 16- to 24-year-old STI-clinic visitors (2009-2015). Vaginal swabs were analyzed using a sensitive PCR-based reverse line blotting system (SPF10-LiPA25) which is able to detect the high-risk types 16/18/31/33/35/39/45/51/52/56/58/59. VE was estimated by a logistic mixed model corrected for demographics and (sexual) risk behavior, with a random intercept to account for residual clustering of HPV types within individuals. HPV DNA positivity was compared between women who reported to be vaccinated at least once and women who reported to be unvaccinated. VE was calculated as (1-adjusted Odds Ratio)*100%.

Results

We included 1087 vaccine-eligible women of the PASSYON study years 2011-2015. Of these women, who were 16- to 22-years-old, 53% tested positive for a high-risk type and 60% reported to be vaccinated. Among women with serum available (43%), the self-reported vaccination status agreed well with the HPV16/18 antibody concentration (AUC 92.3%), suggesting reliable reporting. The pooled VE against the vaccine types HPV16/18 was 89.9% (81.7-94.4); 92.3% (82.5-96.6) against HPV16 and 85.5% (66.0-93.8) against HPV18. Moreover, we calculated significant VE against the non-vaccine types HPV45 (91.0% [59.7-98.0]), HPV35 (57.1% [2.3-81.2]), HPV31 (50.0% [10.8-72.0]) and HPV52 (37.2% [9.2-56.6]). Vaccinated women were more often HPV59 positive (6.0%) than unvaccinated women (3.4%), resulting in a VE of -89.4% (-259.9-0.3). The pooled VE against all high-risk types was 32.9% (20.2-43.7).

Conclusion
We demonstrate a high VE against prevalent infection with the bivalent vaccine types. In addition, we found significant cross-protection against HPV types 45, 35, 31 and 52. The negative VE against HPV59 is notable and needs further investigation.
PILOT STUDY ON USE OF INNO-LIPA® HPV GENOTYPING EXTRA II WITH COLLI-PEE COLLECTED UCM PRESERVED URINE

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Background / Objectives

The INNO-LiPA HPV Genotyping Extra II assay can individually detect 32 HPV genotypes. Performance of this assay has been demonstrated on cervical scrapes, but no data are currently available regarding HPV DNA detection in first void urine. The aim of this pilot study is to determine whether the INNO-LiPA HPV Genotyping Extra II test is compatible with self-collected first void urine specimens.

Methods

18 Colli-Pee™ collected, preserved first void urine samples (16 HPV positive samples previously identified with the Riatol qPCR HPV genotyping assay (AML) and Multiplex HPV Genotyping assay (Diamex)) were analysed – samples originated from a cohort of women with self-reported prior HPV positive test results.

The participants collected the first void urine self-sample at home and sent the collection vial containing preservative uncooled by postal mail to the Antwerp University. Prior to the PCR tests 4 ml of urine/UCM mixture was concentrated on an ultrafiltration membrane and extracted with easyMAG® (bioMérieux).

Results

17/18 urine samples were successfully genotyped with the INNO-LiPA HPV Genotyping Extra II assay. An opened vial in the PCR instrument caused an invalid result for one sample. Two samples were HPV DNA negative as found by the Riatol qPCR HPV genotyping assay. For 13 out of the 15 remaining samples at least one high risk HPV type was detected by INNO-LiPA HPV Genotyping Extra II assay and confirmed by one or two of the other assays.

Conclusion

These preliminary results confirm that the INNO-LiPA HPV Genotyping Extra II assay is compatible with self-collected first-void urine. Confirmation of performance by testing larger series in a clinical setting is warranted.

References

EVALUATION OF BD ONCLARITY IN DETECTION OF CANCER AND PRE-CANCER IN WOMEN WITH ASCUS/LSIL IN CHINA

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Background / Objectives

About 30~40% ASCUS women and 80% LSIL women are high risk HPV (HR-HPV) positive, while the prevalence of CIN2+ is about 3~9% and 15%, respectively. This indicated that there may be clinical benefit in stratifying HR-HPV positive women with ASCUS/LSIL by HPV genotypes. In this study, we evaluated the clinical performance of onclarity HPV test for partial HPV genotypes among women with ASCUS/LSIL cytology.

Methods

320 (221 ASCUS and 99 LSIL) women with cytological ASCUS/LSIL were recruited in the study. All those participants were referred colposcopy and directed biopsy, four-quadrant cervical biopsy was conducted when no visible lesions were found under colposcopy. All of the cervical samples were tested by cobas HPV test and onclarity HPV test with nine typing channels: HPV16, HPV18, HPV31, HPV45, HPV51, HPV52, HPV33/35, HPV35/39/68 and HPV56/59/66.

Results

The agreement rate between cobas HPV test and onclarity HPV test was satisfactory for testing 14 types HR-HPV, HPV16, HPV18 and HPV non-16/18, as for positivity agreement rate and kappa value, those two tests also showed preferably performance except HPV 18. BD onclarity HPV test can provide more HPV genotypes information that can be used for evaluation of ASCUS/LSIL triage. On the basis of HPV16/18, other HPV types by their risk grade were sequentially added into 6 groups, marked as subG1 (HPV16/18/31), subG2 (HPV16/18/31/33/35/58), subG3 (HPV16/18/31/33/58/35/39/68), subG4 (HPV16/18/31/33/58/39/68/35/52), subG5 (HPV16/18/31/33/58/39/68/35/52/45) and subG6 (HPV16/18/31/33/58/39/68/35/52/45/51). We found that the AUC increased when HPV31, HPV33/58, and HPV35/39/68 were added into the groups (AUC: subG1=0.687, subG2=0.746, and subG3=0.755), while it decreased when HPV52, HPV45, HPV51, and HPV59/66/68 were added into other groups (AUC:}
subG4=0.748, subG5=0.746, subG6=0.743, and pooled 14 HR-HPV=0.709). AR (absolute risk) of CIN2+ was lower among women with HPV52/45/51/66/68 positive than that of pooled 14 HR-HPV types positive in cytological ASCUS/LSIL women [17.1% (95%CI: 9.93%-27.8%) vs. 35.0% (95%CI: 28.0%-42.8%)]. Compared with women with HPV52/45/51/66/68 positive, the risk of CIN2+ among women with HPV 16/18, HPV31, and HPV33/58 positive were 3.35 (95%CI:1.89-10.1), 2.43 (95%CI:1.04-5.66) and 2.22 (95%CI: 1.17-4.23), respectively.

Conclusion

The management of women with HPV 16/18/31/33/58 positive should be different from women with other 9 types HPV positive in China. Women with cytology ASCUS/LSIL and HPV 16/18/31/33/58 positive referred to colposcopy seemed to be of better clinical performance.
ANALYTICAL STABILITY OF SUREPATH COLLECTED CERVICAL SMEAR SAMPLES FOR HPV TESTING

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Background / Objectives

Changing from cytology to HPV testing as the primary screening analysis in organized programs for Cervical Cancer Screening will increase the requirements for bio-banking of cervical screening samples with respect to audit and quality assurance. The quality of stored, extracted DNA is well documented but what about storage of original sample material? The cervical cancer screening in Denmark is predominantly conducted using SurePath (BD, Sparks, Maryland, US) for sample collection. Here we present data on the analytical stability of SurePath collected cervical samples for HPV testing with ≥7 months storage of original SurePath samples between baseline testing and re-test.

Methods

We collected 1216 samples (897 NILM and 319 ≥ASCUS samples) from Danish women undergoing routine screening in the Capital Region of Denmark. The samples were split into two aliquots. Aliquots were tested at base line and after ≥7 months (4°C) using the clinical validated Onclarity HPV assay (BD, Sparks, Maryland, US). Stability of the SurePath samples were assessed by 1) clinical reproducibility of results between 1st and 2nd test, 2) analytical quality assessment using the mean Ct-value of the internal control (HBB) of the Onclarity assay as a proxy-marker of overall DNA quality after storage, and 3) HPV genotype specific Ct-values of positive samples were used to address the stability of HPV DNA. The mean Ct-values of the internal HBB control and the HPV positive results were compared using the one-way ANOVA test (SPSS version 22).

Results

The overall reproducibility (positive-positive and negative-negative) was 98.0% (N=1192) with 1.8% (N=24) being discordant. No significant difference in the mean Ct-values of the internal HBB control (HBB; p=0.667) between the baseline test and the 2nd test were observed. The discordant samples were Ct 32.1±1.4 versus the manufacturer defined cut off of Ct 34.2. Furthermore, no significant difference were observed in measured Ct-values of the individual HPV genotypes detected between baseline and 2nd test; HPV16; p= 0.773, HPV18; p=0.530, HPV31; p=0.701, HPV33/58; p=0.996, HPV35/39/68; p=0.923; HPV45; p=0.992, HPV51; p=0.722, HPV 52; p=0.896, HPV56/59/66; p=0.626.
Conclusion

In conclusion, SurePath collected cervical screening samples can be stored at 4°C for at least 7 months without significant deterioration of the clinical or analytical quality of the material with respect to HPV testing when using the Onclarity HPV assay.
FC 10-04
VALGENT-4 CLINICAL VALIDATION OF THREE HPV GENOTYPING TESTS ON SUREPATH SCREENING SAMPLES FROM THE DANISH CERVICAL SCREENING PROGRAM

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Background / Objectives
As the demand for human papillomavirus (HPV)-related cervical screening increases, novel HPV tests must be evaluated using well-annotated samples. The Validation of Human Papilloma virus (HPV) Genotyping Tests (VALGENT) framework is an international collaboration designed to facilitate the clinical validation and comparison of HPV assays that offer genotyping capabilities. Here we present the data from three assays; BD Oncology HPV assay (BD), Genomica CLART4s (CLART) and Agena MassArray HPV assay (MA).

Methods
In total, the Valgent4 consists of 1000 consecutive screening samples and an enriched subset of 300 samples equally divided between samples with ASCUS, LSIL and HSIL, all collected in SurePath LBC medium from Danish routine cervical screening. Briefly, the Oncology assay is a real time PCR assay that detects 14 HR-HPV genotypes in nine groups (16,18,31,45,51,52,33/58, 56/59/66,35/39/68), the CLART4 assay is a PCR-based Microarray full genotyping assay that detects 36 genotypes (the 14 HR-HPV types and 22 non-HR HPV types), and the MA is a full genotyping MALDI-TOF-based assay that detects 19 genotypes (the 14 HR-types and 5 non-HR-types). Here, the analysis is limited to HR-HPV genotypes only.

Results
Oncology, CLART4 and MA detected 371, 455, and 514 positives samples respectively. 947 samples had normal cytology, 106 ASCUS, 121 LSIL and 124 ≥HSIL. Histological follow-up at 12 months after initial sample collection resulted in 118 normal histology results including 29 ungraded CIN, 43 CIN1, 31 CIN2, 64 CIN3 and 7 cancers. The assays showed overall good pairwise HR-HPV detection concordance of 87-92%. At individual HR-HPV genotype level, the concordance varied from 40-84% (Oncology vs MA), 32-62% (Oncology vs CLART4) and 32-66% (CLART4 vs MA). HPV16, 18 and 31 had the highest pairwise assay concordance. For samples with ≥CIN2 outcome, the pairwise assay concordance was higher for
almost all genotypes detected by the three assays. The sensitivity for detection of \( \geq \text{CIN2} \) was 95%, 98% and 96% for Onclarity, CLART4 and MA, respectively.

**Conclusion**

The three genotyping assay had overall good concordance at HR-HPV level, whereas on genotype level the discordance became noticeable. Genotypes HPV16, 18 and 31 had the highest pairwise concordance between the three assays. Comparison with a validated standard comparator test, planned later, will allow verification whether these tests fulfill the requirements for use in cervical cancer screening.
OPTIMIZATION OF THE RIATOL QPCR HPV GENOTYPING ASSAY BY CHOOSING A THRESHOLD ASSURING SATISFACTORY ACCURACY TO DETECT HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background / Objectives

To identify the optimal viral load threshold of the in-house AML Laboratory RIATOL qPCR HPV genotyping assay (qPCR) (Antwerp, Belgium) assuring satisfactory accuracy to detect high grade cervical intraepithelial neoplasia (CIN2+).

Methods

The clinical accuracy of the qPCR to detect CIN2+ was assessed using a set of cervical samples compiled for the VALGENT-3 project. The VALGENT framework is designed to assess the analytical and clinical performance of HPV tests that offer limited to extended genotyping capability. “VALGENT-3” panel comprised 1,600 samples from Slovenian women aged 20-64 years (1,300 sequential cases from routine screening and 300 “enriched” abnormal samples – 100 HSIL, 100 LSIL and 100 ASC-US).

The VALGENT-3 panel contained 126 specimen of women with CIN2+ (used to assess sensitivity) and 1,167 specimen from women with 2 consecutive negative Pap smears (used to assess specificity). Performance relative to the Hybrid Capture 2 (HC2) was also analyzed as per the non-inferiority criteria defined by Meijer et al. in 2009. The trade-off between sensitivity and specificity with different viral load cutoffs was assessed by ROC curve analysis. The cumulative hrHPV load was defined as the logarithm of the sum of the type-specific loads of the 14 HPV types.

Results

The qPCR had a sensitivity and specificity for CIN2+ of 97.6% (CI: 93.2-99.5%) and 85.1% (CI: 82.9-87.1%) respectively when the lowest analytical cutoff was used. At a cutoff of 1.58 log copies/cell, qPCR had a sensitivity of 96.0% (CI: 91.0-98.7%) and a specificity of 89.5% (87.6-91.2%). At this cutoff, accuracy of the qPCR was non-inferior to the HC2: relative sensitivity of 1.00 [CI: 0.97-1.03 (p<0.001)] and relative specificity of 1.00 [CI: 0.98-1.02 (p<0.001)].
Conclusion

HPV tests that provide viral load measurements (or other quantifiable signals) allow flexibility to optimize accuracy required for use in cervical cancer screening.
EVALUATION OF XPERT®HPV IN CERVICAL SPECIMENS COLLECTED IN SUREPATH PRESERVATIVE FLUID: AN INTERIM ANALYSIS

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Background / Objectives

Currently, a large scale study is being conducted to verify compatibility of the Xpert®HPV assay (Cepheid) with specimens collected in Surepath Preservative Fluid. Primary aim of this study is to investigate whether both fractions (primary cells and cell pellet) can be considered as suitable input material for the Xpert HPV assay. For this interim analysis, endpoints are limited to results with clinical cutoff only, and matched to corresponding outcomes of the Riatol qPCR genotyping assay as standard comparator test (limited to HR-HPV types only).

Methods

In total, 100 samples were prospectively collected and processed according to the standard Surepath method for cytology. Two different cell fractions were obtained per sample, i.e. left-over primary sample and pelleted cells after gradient purification. Both cell fractions are stored at 4°C, and further processed on both the Xpert HPV assay and the Riatol qPCR genotyping assay. Clinical thresholds were applied accordingly during this analysis. Paired T-test was used to compare Ct-values of both fractions.

Results

Median age of study participants was 46 years (IQR = 31 – 56). The first 100 samples gave valid results for 96 paired samples on Xpert HPV. HPV prevalence in this subset was 27/96 (28.1%). In all tested sample pairs, no clinical difference was observed between primary sample and pelleted cells. When comparing Ct-values for HMBS (cell adequacy control), significantly higher HMBS signals were found in the primary sample versus the processed fraction (p < 0.001), indicating less cells in this fraction. At clinical level, full concordance between the standard comparator test (Riatol qPCR genotyping assay) and the Xpert HPV assay was observed. Errors (4 errors/200 reactions; 2%), were observed both in primary sample fraction (n=3) and in pelleted cells (n=1).

Conclusion
This interim analysis indicates a good compatibility of the Xpert HPV assay with samples collected in Surepath Preservative Fluid. Both cell fractions, generated after standard Surepath processing, can be analyzed without pre-treatment on the Xpert HPV assay. Results are shown to be clinically valid, as no differences could be observed with a validated comparator assay (Riatol qPCR genotyping assay). Higher concentration of cells was measured in the pelleted fraction, however these preliminary results do not indicate influence at clinical level, as no discordant HPV results between both Surepath cell fractions could be observed.
REPRODUCIBILITY OF HUMAN PAPILLOMA VIRUS TYPING WITH XPERT REAL-TIME PCR ON ARCHIVAL CYTOLOGY SAMPLES


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Background / Objectives

Since most cervical cancers are HPV-associated, primary cervical screening is changing to HPV test in many European countries. HPV testing techniques are mainly based on direct DNA hybridization or nucleic acid amplification. HPV DNA testing may be performed directly from residual liquid-based cytology (LBC) specimens. In some cases, particularly in retrospective studies or for quality control purposes, previously archived samples may be used. In these cases, DNA degradation may become a potential issue. The aim of this study was to evaluate the reproducibility of HPV typing on archived LBC-specimens with the Real Time PCR (RT-PCR)- based Xpert® HPV assay (Cepheid, Sunnyvale, USA).

Methods

A total of 150 LBC samples (ThinPrep, Hologic, Inc. Marlborough, MA-USA) with a previous positive HPV test with Hybrid Capture II (HCII, Qiagene GmbH, Hilden-Germany), were included in the study and divided in 3 groups. Group 1 included 50 samples that were typed with the xpert assay immediately after the positive HCII test. Group 2 and Group 3 included 50 cases each with a previous positive HCII test which had been carried out 12 and 36 months before, respectively.

The observed agreement between Xpert® HPV assay and the previous HCII test was 96% for the first Group, 90% for the second Group and 94% for the third Group. The observed agreement didn’t differ significantly among the tree groups (p = 0.606).

Conclusion

These results show that HPV-typing by the RT-PCR based Xpert assay is reproducible even in long-term stored archival LBC-specimens. This may be relevant particularly for retrospective studies or for quality control purposes.
FC 10-08
DEVELOPMENT OF A NOVEL MULTIPLEX TYPE-SPECIFIC QUANTITATIVE REAL-TIME PCR FOR DETECTION AND DIFFERENTIATION OF INFECTIONS WITH HPV2, HPV27, AND HPV57

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Background / Objectives

HPV types HPV2, HPV27, and HPV57 are etiologically associated with more than 65% of common warts (verrucae vulgares), the most frequent HPV-associated benign lesions of the skin. When common warts appear in the anogenital region they can be misdiagnosed as anogenital warts (condylomata acuminata), which are etiologically associated with HPV6 and HPV11. In children the mentioned misdiagnosis could have potentially serious legal consequences, since the appearance of novel wart(s) in a child’s anal or genital region can be considered as an indicator of sexual abuse and can potentially trigger legal action against the parents or household members. Thus, although routine detection of HPV types present in tissue specimens or swabs of common and anogenital warts is not generally recommended, it could be very helpful in some clinical circumstances and/or for legal purposes, especially in children. To the best of our knowledge, no quantitative real-time PCR (RT-PCR) allowing simultaneous amplification and differentiation of HPV2, HPV27, and HPV57 has been developed so far. The present study describes the development and evaluation of the first multiplex type-specific quantitative RT-PCR, enabling simple, rapid, sensitive, and specific concurrent detection and differentiation of HPV types HPV2, HPV27, and HPV57 in a single PCR reaction.

Methods

The novel HPV2/27/57 multiplex RT-PCR was designed and optimized on plasmid standards and clinical samples of common warts.

Results

The HPV2/27/57 multiplex RT-PCR with a dynamic range of seven orders of magnitude (discriminating 10 to 10^8 viral genome equivalents/reaction) has an analytical sensitivity of at least 10 viral copies of each targeted HPV type/reaction, and no cross-reactivities were observed among the included targets. All three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of 10^8, 10^7, 500, 100, and 10 copies of non-targeted viral DNA/reaction, and the performance of the HPV2/27/57 multiplex RT-PCR was additionally not affected by the presence of background human genomic DNA. When testing DNA isolates obtained from fresh-frozen tissue specimens of various
children’s warts, the results of the HPV2/27/57 multiplex RT-PCR were completely in line with the results of the conventional low-risk Alpha-PV PCR.

**Conclusion**

The newly developed HPV2/27/57 multiplex RT-PCR is an appropriate test for use in routine clinical laboratory settings and for studies focusing on the molecular epidemiology, pathogenesis, and natural history of HPV2/27/57-related lesions.
THE 5-YEAR INCIDENCE AND CLEARANCE OF TYPE-SPECIFIC HPV IN A SCREENING COHORT IN CHINA

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Background / Objectives

We aimed to evaluate the 5-year incidence and clearance of type-specific high-risk HPV (hr-HPV) among a Chinese rural women cohort.

Methods

A screening cohort with 1,997 women aged 35-45 years was followed up with an interval of approximate 5 years. HPV genotyping (INNO-LiPA Extra, Innogenetics, Belgium) was performed on cervical samples collected from HPV positive women (Hybrid Capture II) in this cohort since 2005. The 5-year incidence and clearance of type-specific HPV and the relevant demographic factors were calculated.

Results

The 5-year overall incidence of hr-HPV was 15.6% and the clearance was 69.0%. HPV 16 related types had two times probability of HPV incidence (10.6%) after 5 years than HPV 18 related types (4.4%) (P<0.001), while the clearance of HPV 16 related types (74.1%) was lower than that of HPV 18 related (85.9%) (P=0.048). The incidence and clearance of HPV 16 was 2.7% and 70.9%. HPV 52 and 16 ranked the top of hr-HPV incidence and HPV 52 and 18 ranked the top of hr-HPV clearance. Sexual debut age was the main factors correlated with HPV incidence, with the adjusted RR of 1.478 (95%CI: 1.110-1.967).

Conclusion

The high incidence, persistence and low clearance prompted the importance of screening in HPV 16 related positive mid-adult Chinese women. Women with HPV 16 related repeated positive within 5 years should be considered as a high risk population for cervical cancer.
HIGH-RISK HUMAN PAPILLOMAVIRUS SCREENING ROLL-OUT IN NORWAY

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Background / Objectives

A shift of primary cervical screening from cytology to hrHPV detection, although only for women aged 34 to 69 years, implies a major shift in the technical infrastructure for screening. To develop real-world evidence for preferred cervical cancer screening strategies, we compared liquid based cytology (LBC) screening every 3 years (current screening modality) with high-risk human papilloma virus (hrHPV) testing every 5 years in Norway (health service study trial number 006_2014_10_RHS).

Methods

Between February 2015 and April 2017, approximately 140,000 women aged 34 to 69 years who returned for their routine, triennial cervical cancer screening were assigned hrHPV-testing (cobas® HPV Test (Roche Diagnostics) or LBC, based on even/odd day of birth. Cervical intraepithelial neoplasia grade 2, 3 and cervical cancer (CIN2+) was detected among 32,434 women who completed their follow-up of a positive screening test by early 2017.

Results

Screening attendance by age was similar in HPV-screening and LBC-screening, being 68% after 1st and 28% after 2nd reminder. The proportion of screening test positives was 5.4% in LBC-screening and 6.5% in HPV-screening, and declined by increasing age. HPV16/18 was detected in 20% of hrHPV-positives. Compared to LBC-screening, we observed 40% more biopsy and/or treatment referrals, 78% more CIN2+ and 50% more CIN3+ in HPV-screening.

Conclusion

HPV-screening was well accepted and detected more pre-cancers, suggesting that HPV-screening should replace LBC-screening. Randomized implementation of HPV-screening allows to monitor the performance of novel technology in real-life, reassuring the overall high performance of the program and mitigating the transition.
EXTENDED SCREENING INTERVALS: EVIDENCE FROM THE ARTISTIC TRIAL COHORT

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Background / Objectives

The UK National Screening Committee (NSC) based its recommendation that HPV testing should replace cytology in primary screening largely on the 2009 follow-up results of the ARTISTIC trial. The NSC must now decide on screening intervals in time for national roll out of primary HPV screening (due 2019). Options include extending the screening interval up to 10 years for HPV negative women and delaying recall for HPV positive women with normal cytology, as their infections are usually transient.

Methods

In the ARTISTIC Trial 24,510 women attending for routine cervical cytology in Greater Manchester in 2001-2003 were recruited. During the trial women were recalled 3-yearly and histology results were obtained from local laboratories. After 2009 histological follow-up and sample collection ended and the women returned to routine cytological screening with recall 3-yearly below age 50 and 5-yearly at age 50-64. We have followed the trial cohort to 2015 through national cancer registration for CIN3 and cancer and through linkage to the cervical screening call-recall system to obtain lifetime cytology records.

Results

The analysis included 24,496 women at round 1 and 13,591 at round 2 (30-48 months later). Follow-up via local histology laboratories and national cancer registration identified 505 cases of CIN3+ (including 22 invasive cervical cancers). The cumulative CIN3+ risk 10 years after a negative HPV test (0.31%, 95%CI 0.18-0.49 in the revealed arm) was similar to that 3 years after negative cytology (0.30%, 95%CI 0.23-0.41 in the concealed arm) and fell sharply with age, from 1.1% below 25 (95%CI 0.7%-1.8%) to 0.08% (95%CI 0.03%-0.20%) above 50.

Conclusion

We found a similar level of protection 10 years after a negative HPV test and 3 years after negative cytology. These data support a much longer screening interval after a negative HPV test than after a negative cytology test.

About three quarters of women with HPV infection and normal cytology clear their infections within about 3 years. Their risk of CIN3+ within this time is low (1.5%),
suggesting that the current policy of annual repeat testing and referral after 2 years is too conservative. Approximately 40% of women who remained HPV positive had cleared their initial infection and acquired a new HPV type. Cumulative CIN3+ risks in women with type-specific persistent infections are about 6 times higher than in women with new infections. Triage strategies based on HPV persistence would therefore reduce unnecessary referral of women with new (and largely transient) infections.
4 - YEAR EXIT RESULTS FOR WOMEN WITH NO CIN2 OR WORSE DETECTED IN EARLIER SCREENING ROUNDS IN THE HPV FOCAL TRIAL


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Background / Objectives

The HPV FOCAL RCT compares liquid based cytology (LBC) with hybrid capture 2® (HC2) triage of ASCUS at entry and 2-years (Control Arm) to HC2 testing with LBC triage at entry (Intervention Arm). Women exit with HC2/LBC co-testing performed at four years in both arms. We examine exit results in subjects without cervical intraepithelial neoplasia or worse (CIN2+) detected in earlier trial screening.

Methods

Subjects were included if they were eligible for routine screening at the time of the exit screen. For the Intervention arm this was women HC2 Negative at baseline (HPVBaseNeg), or HC2 positive and negative for intraepithelial lesion or malignancy (NILM) at baseline and HC2 negative at 6-12 months retesting (HPVRev), or recommended for colposcopy where no CIN2+ was detected (HPVColpoNeg). For the Control arm this was women NILM or atypical cells of undetermined significance (ASCUS) and HC2 negative at baseline and at two years (CYTNeg), recommended for colposcopy at baseline and no CIN2+ found (CYTColpoNeg0) or recommended for colposcopy at 2-years, but not at baseline, and no CIN2+ found (CYTColpoNeg2). Results presented are based upon exit (4-year) co-testing where women were referred to colposcopy if HC2 positive or ASCUS or worse.

Results

9,552 women were randomized to the Intervention arm and 8,338 were eligible and attended the exit screen, 9,457 women were randomized to the Control arm and 7,424 were eligible and attended the exit screen: the breakdown of subjects is given in the attached table. The overall relative risk of CIN2+ at exit for Intervention versus Control was RR=0.86 (95%CI=0.58-1.26). For those with a single negative HPV tests versus those with two consecutive negative LBC tests (i.e. HPVBaseNeg versus CYTNeg) the relative rate at exit contesting was RR=0.69 (0.45-1.07). Women having earlier negative colposcopy were at elevated risk in both arms compared to others eligible for routine exit screening: Intervention Arm RR=10.0 (5.5-18.3); Control Arm RR=5.0 (2.1-11.5).

Table: Rates of CIN2+ Identified at 48 Month Exit Cotesting by Study Arm Subgroup

<table>
<thead>
<tr>
<th>HPVBaseNeg</th>
<th>HPVRev</th>
<th>HPVColpoNeg</th>
<th>CYTNeg</th>
<th>CYTColpoNeg0</th>
<th>CYTColpoNeg2</th>
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<tr>
<td></td>
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<tr>
<td>Number</td>
<td>7869</td>
<td>166</td>
<td>303</td>
<td>7308</td>
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<td>--------</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>CIN2+</td>
<td>35</td>
<td>2</td>
<td>14</td>
<td>47</td>
<td>5</td>
</tr>
<tr>
<td>Rate/100 (95%CI)</td>
<td>0.44 (0.32-0.61)</td>
<td>1.2 (0.33-4.3)</td>
<td>4.6 (2.8-7.6)</td>
<td>0.64 (0.48-0.75)</td>
<td>4.6 (2.0-10.4)</td>
</tr>
</tbody>
</table>

**Conclusion**

Women recommended for colposcopy with no significant lesion detected were at elevated subsequent risk and their careful surveillance is indicated.

**References**

Ogilvie, G et al, HPV for cervical cancer screening (HPV FOCAL): Complete Round 1 results of a randomized trial comparing HPV-based primary screening to liquor-based cytology for cervical cancer, IJC 140 (2017):440-448
FC 11-04
CANCER CASES IDENTIFIED IN A RANDOMIZED IMPLEMENTATION OF PRIMARY HPV-TESTING IN THE NORWEGIAN CERVICAL CANCER SCREENING PROGRAMME

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Background / Objectives

High risk Human Papilloma Virus (HPV) testing is currently implemented in a randomized controlled fashion as the primary test in the Norwegian cervical screening programme. We present detailed evaluation of the cancer cases identified.

Methods

The implementation involves women in the age-group 34-69 years in four Norwegian counties, counting approximately 285,000 women. The follow-up algorithm after abnormal HPV-test is more aggressive than for abnormal cytology, and more women are referred to immediate biopsy, and thereby potentially earlier detection of cancers. To compare symptomatic and screening detected cancer cases among those allocated to HPV test or cytology, we included women with at least 15 months follow-up since screening. Description of screening results (cytology/HPV status/genotype), screening history, symptoms, FIGO-stadium and age of the cancer-diagnosed women are presented.

Results

By March 2016, approximately 140,000 women have been screened, half with HPV test and half with cytology. Around 32,000 women have had adequate follow-up time. A total of 25 cancer cases were identified; 14 cases among HPV-screened (12 squamous cell carcinoma, 2 adenocarcinoma) and 11 among cytology-screened (9 squamous cell carcinoma, 1 adenocarcinoma, 1 other cervical cancer type). 86% of the cancer cases was diagnosed in women below 50 years after primary HPV test compared to 46% in the cytology group. More than 50% of the women diagnosed with cancer were screened sub-optimally. Around 80% of the cancers were related to HPV16 and HPV18, and the majority of the cancers were FIGO stadium I. Updated results will be presented at the conference.

Conclusion

As we expected, observed number of cancer cases were comparable in HPV-screening and cytology screening, suggesting high performance of HPV-testing in routine screening.
DETECTION OF CIN2+ IN WOMEN WITH NORMAL CYTOLOGY USING A 3-TYPE HPV mRNA TEST

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Background / Objectives

Despite a well-established cervical cancer screening program in Norway, the incidence of CxCa in young women is increasing, peaking at 35 years. Twenty five percent of all women diagnosed with CxCa had normal cytology within 3 years of cancer diagnosis. We wanted to estimate the detection rate of CIN2+ in women with normal Pap smears by rescreening Pap smears from HPV mRNA 16, 18, and 45 positive samples. HPV 16, 18 and 45 cause 90% of cervical cancers in young women.

Methods

From April 2016, the Department of Pathology, University Hospital of North Norway, introduced a study by rescreening all normal Pap smears that had a positive HPV mRNA test (PreTect SEE). Women with revised cytology were followed up according to national guidelines.

Results

Of 26,948 women with Pap smear, 184 (0.7%) had normal cytology and a positive HPV mRNA test. After rescreening of the index cytology, 63 women had abnormal cytology. At present 42 women have had colposcopy, resulting in 7 women with normal biopsies, 21 CIN1, 9 CIN2 and 5 CIN3. The positive predictive value of CIN2+ among women with biopsy was 33.3 % (14/42).

Conclusion

By testing all women with normal cytology with a specific HPV mRNA test, an increase in screening program sensitivity can be achieved. When more women with CIN2+ are detected in the first screening round, fewer women will develop cervical cancer before next screening. The volume of rescreened smears (0.7%) is very low but adds significant improvement of screening sensitivity and increases quality in educating the screeners by rescreening presumably false negative Pap smears.
THE CLINICAL AND ECONOMIC IMPACT OF HPV EXTENDED GENOTYPING FOR THE INDIVIDUALIZED RISK MANAGEMENT OF PATIENTS: RESULTS OF AN ECONOMIC MODEL

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Background / Objectives

Denmark has a well-established cervical cancer screening program with nationwide screening guidelines issued by the National Board of Health. In women aged 30–59 years, guidelines recommend cytology-based screening with high-risk HPV testing for ASC-US triage. In women aged 60–64, primary HPV testing is recommended. Current algorithms utilize pooled HPV assays where either no HPV genotypes or only types 16/18 are differentiated. New technologies are available which can differentiate all 14 high-risk HPV types, potentially offering greater ability to stratify patients based on individual risk.

In this study, a health economic model was developed to estimate the impact of adopting extended HPV genotyping within the Danish cervical cancer screening program. Current screening strategies using no or partial HPV genotyping were compared with strategies using extended genotyping in terms of number of colposcopies and CIN2+ detection.

Methods

A budget impact model was constructed in Excel using data from the published literature on population size, HPV prevalence and disease outcomes. For ages 30–59 years, we compared 1) HPV triage of ASC-US without genotyping with 2) HPV triage of ASC-US using extended genotyping. For ages 60–64 years, we compared 1) HPV primary screening using HPV16/18 genotyping with 2) HPV primary screening using extended genotyping. In the extended genotyping algorithms, women with HPV16, 18, 31, 33, 45, 52, or 58 were sent to colposcopy, while women with lower risk genotypes (35, 39, 51, 56, 59, 66, or 68) were sent to a 1-year follow-up.

Results

Preliminary analyses indicated that using extended HPV genotyping for ASC-US triage in women aged 30–59 would reduce the number of colposcopies by 29.7%, at the cost of a slight (4.8%) increase in the number of CIN2/3 cases referred to 1-year follow-up instead of immediate colposcopy. Extended genotyping for HPV primary screening at
ages 60–64 would reduce colposcopies by 13.9%, while slightly (4.6%) increasing the number of CIN2/3 cases referred to 1-year return instead of immediate colposcopy.

Conclusion

Extended HPV genotyping may potentially reduce colposcopies in the Danish population with minimal sacrifice for disease detection. The CIN2/3 cases referred to 1-year return in the extended genotyping algorithms were attributed to lower risk HPV genotypes and likely had low probability of progressing to cancer within a year. Further analyses will be presented at the conference, including the budgetary impact of extended genotyping and the performance of extended genotyping for HPV primary screening of women aged ≥30.
FC 11-07
HPV Primary Screening Pilot Study: molecular testing of potential triage strategies for HPV-positive women


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Background / Objectives

The objective of this study is to evaluate and compare different strategies for the triage of women with a HPV-positive primary screening test. Clinical performance in terms of sensitivity, specificity, PPV, NPV will be calculated both cross-sectionally and longitudinally for each triage strategy. The overall aim of the study is to define optimal algorithms for triage of HPV DNA positive women from primary HPV screening.

Methods

In partnership with CervicalCheck, The National Cervical Screening programme, CERVIVA are undertaking a longitudinal observational HPV primary screening study which will evaluate different triage strategies for management of a HPV-positive primary screening test. Cervical cytology samples from approximately 13,000 women undergoing routine cervical screening will be tested for HPV DNA (cobas 4800 HPV test) and mRNA (Aptima HPV assay). All HPV-positive women will be further assessed with cytology and a panel of molecular tests including HPV16/18 genotyping, p16INK4a/Ki-67, and specific methylation markers. The performance of different triage strategies will be examined both cross-sectionally and longitudinally over two screening rounds for detection of CIN3+.

Results

To date 8500 woman have been recruited into the study. The median age of the population is 39 years. HPV DNA testing, performed on 7301 samples, shows a 14.7% positivity rate. HPV mRNA, performed on 7394 samples, gave a 12.7% positive rate. HPV mRNA had a significantly lower positivity rate in women under the age 40 years and women with a negative cytology (p=0.001 and p=0.0015). Second round testing identified 32% of HPV positive women were positive for HPV 16/18 and 30% had an abnormality on cytology. In a smaller subset 38% were positive for p16/Ki-67.

Conclusion
Overall prevalence of HPV mRNA is lower than HPV DNA in the study population. Here we present the preliminary cross-sectional data in relation to each of the putative triage tests.
GENOTYPING AND CYTOLOGIC TRIAGE OF HPV POSITIVE WOMEN FOR THE DETECTION OF CERVICAL HIGH-GRADE LESIONS

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Background / Objectives

The VASCAR (viral testing alone with Pap triage for screening cervical cancer in routine practice) study was a single-center demonstration project initiated in Montreal, Canada in 2011 among more than 23,000 women attending routine cervical cancer screening. In a secondary phase of VASCAR, we determined genotype-specific risks of disease progression to biopsy-confirmed cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) associated with HPV types 16, 18, and/or the other 12 (pooled) high-risk types, compared to Pap cytology. We also assessed the diagnostic performance of HPV genotyping compared to cytological triage.

Methods

Women aged 30-65 were originally screened for HPV using the Hybrid Capture® 2 (HC2) Test. Women with positive results were triaged using conventional cytology, and those with positive Pap cytology results (≥ASC-US; atypical squamous cells of undetermined significance) were referred to colposcopy. We retrospectively genotyped 1396 cervical specimens that were HPV+ with HC2 using the Roche’s cobas® 4800 HPV system, and extracted the women’s medical history. We evaluated diagnostic performance of triage tests in the first year of follow-up among women positive for: (1) HPV16; (2) HPV18; (3) HPV16 and/or HPV18 and; (4) one or more of the other 12 HPV types. Using hierarchical and exclusive categories of HPV positivity (any HPV16; else HPV18; else 12 other HPVs), we correlated HPV status at enrollment with detection of histologically confirmed CIN2+ by estimating hazards ratios (HR) with 95% confidence intervals (CI) using Cox proportional hazards regression.

Results

Of the 1396 women, 1092 (78%) were classified as normal, 136 (10%) had CIN1, 80 (6%) had CIN2, 81 (6%) had CIN3 and 7 women had cancer, throughout the entire follow-up period. Sensitivity of HPVs 16, 18, 16 and/or 18, and any high-risk HPV for prevalent CIN2+ (n=76) were 35.5% (CI:24.9-47.3), 9.2% (CI:3.8-18.1), 43.4% (CI:32.1-55.3), and 64.5% (CI:52.7-75.1), respectively. Conversely, cytology triage
(ASC-US+) had a sensitivity of 92.0% (CI:83.4-97.0). Corresponding specificity values were 84.0% (CI:81.9-86.0), 95.0% (CI:93.7-96.1), 79.7% (CI:77.4-81.8), 33.4% (CI:30.9-36.0), and 73.6 (CI:71.1-76.0). Compared to cobas HPV- and HC2 HPV+ women, the HRs were 7.3 (CI:3.8-14.3), 3.9 (CI:1.5-10.2), and 2.7 (CI:1.4-5.2) for women with any HPV16, HPV18, and 12 other types, respectively. Compared to women with normal cytology, the HRs for AS-CUS, LSIL, and HSIL (SIL: squamous intraepithelial lesion) were 3.7 (CI:2.5-5.7), 5.0 (CI:3.1-8.0) and 16.5 (CI:11.0-24.7), respectively.

**Conclusion**

Cytology and genotyping seem to be comparable in triaging women with positive HPV results on screening.
FC 11-09
5-TYPE HPV MRNA NEGATIVE WOMEN IN TRIAGE OF ASC-US/LSIL MAY RETURN TO SCREENING AT 3-YEAR INTERVAL – AN HISTORICAL PROSPECTIVE COHORT STUDY

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Background / Objectives

To compare the risk of CIN3+ among women who had a normal cytology (non-exposed cohort) at study start with women who had an HPV mRNA negative ASC-US/LSIL in triage (exposed cohort).

Methods

After exclusion of women who had a previous history of CIN1+ and HSIL, we identified 1063 women who had an HPV negative triage of ASC-US/LSIL over the years 2006 through 2011, and a control cohort of 25 948 women who had a normal cytology during 2006/2007. All women, aged 25-69 at study start, were residents of the counties Troms and Finnmark, Norway, and were followed through December 31, 2014. The HPV test targeted E6/E7 mRNA from the types HPV16, 18, 31, 33 and 45 (PreTect HPV-Proofer, PreTect AS). All analysis were done in SPSS version 24.0 with Chi-square test, T-test and survival analyses.

Results

The exposed cohort were significantly younger than the non-exposed cohort. The crude cumulative proportion of CIN3+ were 2 and 8 per 1000-w.-yrs. at 42 and 78 months of follow-up for the non-exposed cohort, and 14 and 26 (95% CI: 9-43) per 1000-w.-yrs. for ASC-US/LSIL-women. The exposed cohort had significant more extensive follow-up than the control cohort. Over the entire study period 20 cervical cancers were diagnosed in the non-exposed cohort (incidence 15.3/100 000 w.-yrs.) compared to none in the exposed cohort.

Conclusion

Women who have a negative mRNA-test for HPV16, 18, 31, 33 and 45 at triage for ASC-US/LSIL have low risk for CIN3 within the first two screening intervals after triage, and may return to screening at 3-year interval
VALIDATION AND IMPLEMENTATION OF A NEXT-GENERATION qPCR DIAGNOSTIC TOOL FOR HUMAN PAPILLOMAVIRUS TYPE 67 SCREENING

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2Laboratory of Molecular Pathology, AML, Antwerp, Belgium; National Reference Centre for HPV, Brussels, Belgium (Belgium)

Background / Objectives

Cervical cancer prevention in the post-cytology era mainly relies on primary HR-HPV screening. Current epidemiological findings have indicated an underestimated role of possible High-Risk HPV (pHR-HPV) types in cervical carcinogenicity and urge for increased availability of genotype-specific information. This could be suggestive for expansion of existing HPV genotyping assays with pHR-HPV types. It is determined pHR-HPV67 is rarely prevalent in cervical cancer, notwithstanding, its close relation to only HR-HPV types. Our objective was to optimize and validate a Next-Generation pHR-HPV67 qPCR assay. These qPCR assays were applied in a HPV67 epidemiological case study.

Methods

A triple-target HPV DNA qPCR assay was developed. The occurrence of cross-hybridisation with other genotypes within the same α9-species was evaluated by testing the assays with confirmed positive samples. Limit of quantification (LOQ) and limit of detection (LOD) were determined by a dilution series of synthetic gene fragments. Reproducibility and repeatability were performed by testing HPV67 positive samples in duplicate series and over different days. The epidemiological case study comprised 273 samples, gathered in April 2017, enriched with 100 samples of each aberrant cytological category (LSIL, HSIL).

Results

The cross-hybridization assay confirmed high specificity for HPV67 of the different sets. A LOQ concentration of 33.21 copies/μL and 12.67 copies/μL was obtained for the assays targeting HPV67E6, E7 and L1 respectively (analogue results for E6 and E7). A concentration of 33.21 copies/μL (E6), 6.33 copies/μL (E7) and 3.17 copies/μL (L1) was still detectable in 85% of the cases (LOD). Belgian women with HSIL had a HPV67E6, E7 and L1 prevalence of 8.16% (95% CI: 4.19%;15.28%), 8.16% (95% CI: 4.19%;15.28%) and 7.14% (95% CI: 3.50%;14.01%), respectively (no significant differences: p>0.05, Chi Square test). In LSILs, a HPV67E6, E7 and L1 prevalence of 12.04% (95% CI: 7.17%;19.51%), 12.96% (95% CI: 7.88%;20.59%) and 9.26% (95%
CI: 5.11%;16.21%) respectively (p>0.05, Chi Square Test) was determined. Within the screening population, a HPV67E6, E7 and L1 prevalence of 2.22% (95% CI: 1.01%;4.71%), 1.83% (95% CI: 0.78%;4.21%) and 1.83% (95% CI: 0.78%;4.21%) was obtained (p>0.05, Chi Square Test).

Conclusion

Slightly divergent but not significant differences in HPV67L1 prevalence were observed when compared to the HPV67E6 and E7 prevalence. Possible explanations are a variation in PCR efficiency or alternative integration via the HPV L1 gene. The multiple-targeting aspect of the Next-Generation qPCR assay led to the more exact detection of HPV67 and can contribute to an increase in accuracy of HPV detection.
PRESENCE OF KOILOCYTOSIS IN LOW-GRADE CYTOLOGY OF hrHPV-POSITIVE WOMEN IS A NEGATIVE PREDICTOR FOR CIN3+

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Background / Objectives

At the beginning of 2017 The Netherlands converted to hrHPV-based cervical cancer screening with cytological triage of positive cases. A strong increase in colposcopy referrals is foreseen most of which seem unwarranted. Thus, reduction of unjustified referrals will have priority. Koilocytosis is considered as a cytopathic effect of a productive HPV infection but the relation with subsequent diagnosis of high-grade Cervical Intraepithelial Neoplasia (CIN) is unclear. The aim of this study was to investigate if the risk for CIN3 or more (CIN3+) differs between hrHPV-positive ASC-US/LSIL with or without koilocytosis and whether the presence of koilocytosis could justify a more conservatively follow-up regime.

Methods

Retrospective cohort study, using data from the nationwide network and registry of histo- and cytopathology in The Netherlands (PALGA). HrHPV-positive ASC-US/LSIL follow-up cytology of 1 201 women was used from the former cytology-based cervical screening programme. Reporting of koilocytosis was assessed as well as detection rates of CIN1 or less, CIN2 and CIN3+, stratified by the presence or absence of koilocytosis. Crude and adjusted odds ratios (ORs) were calculated.

Results

Koilocytosis was present in 40.1% of hrHPV-positive ASC-US and 45.9% of hrHPV-positive LSIL. CIN3+ is significantly less often found when koilocytosis was reported (7.8% for hrHPV-positive ASC-US with koilocytosis versus 15.8% without koilocytosis). For hrHPV-positive LSIL this was 11.7% versus 20.2%. The crude and adjusted ORs for CIN3+ were 0.45 for hrHPV-positive ASC-US and 0.52 for hrHPV-positive LSIL.

Conclusion

The presence of koilocytosis is a negative predictor of CIN3+. The risk of hrHPV-positive ASC-US combined with koilocytosis for CIN3+ is in the same range as
hrHPV-positive/cytology negative cases and these cases could be followed conservatively by repeat cytology after 6 months. However, the results of this study should be confirmed by the first data derived from the new HPV-based screening programme.
MEASURING CYTOLOGY REPRODUCIBILITY IN THE NEW DUTCH CERVICAL SCREENING PROGRAM

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Background / Objectives

The shift from primary cytology screening to HPV-screening with cytology triage is expected to influence the performance of cytology reading. We initiated an educational program prior to the start of the renewed screening program to refresh classification criteria.

Methods

Two partially overlapping cytology sets of 100 liquid-based cytology slides (ThinPrep) were collected, derived from a blinded co test pilot done previously (the DuSC study), stratified for age and anticipated percentage of abnormalities (i.e. 30%). After examination by an expert panel of 3 cytotechnologists and a pathologist a consensus diagnosis was determined. The sets were ring-studied in the 5 screening laboratories. The first set was followed by an adjudication session with each lab by the national reference official, in which results were discussed and individual classifications were aligned to classification guidelines and consensus diagnosis. Then, after a washout period, a second set of 100 cases with 50 overlapping cases was offered to the same laboratories. Cytotechnologists were asked to individually examine all slides and score them according to KOPAC and Bethesda classification. Pathologists examined only the non-NILM cases.

Results

Discrepancies in cytology reading were examined between i) NILM (especially reactive cellular changes) and ASCUS and vice versa: cases with few atypical changes (< 5 cells per slide) were regarded as difficult for classification of NILM versus ASC-US. Notably, distinguishing between NILM and ASC-US is important because it is the threshold for referral to a gynaecologist in the new HPV-screening program; ii) cytotechnologists and pathologists: pathologists showed moderately higher scores of classifications in the non-NILM’s, iii) laboratories, iv). Missed LSIL/HSIL cases. Wide variation in classification was found in sporadic cases due to technical or obscuring factors (dark staining, thick cell groups, or low cell counts).
Guidance was given on the criteria of the Bethesda classification and according to the company guidelines.

Conclusion

The results show considerable variation in cytology classification between cytotechnologists and pathologists and between laboratories. These results are the starting point of an ongoing external quality control (EQA) and educational program on standardization and optimal cytology classification results within the Dutch cervical screening program. The participants evaluated the learning sets as an important educational tool.
Background / Objectives

Background - The FAM19A4/miR124-2 methylation test (QIAsure Methylation Test, QIAGEN) is a novel assay, designed by Self-screen, that can be used for the triage testing of HPV-positive women or women with ASC-US. The test is a quantitative methylation-specific PCR that detects hypermethylation of the genes FAM19A4 and hsa-mir124-2 in cervical scrapes and self-collected samples. Within the framework of the VALID-SCREEN (H2020) project, the performance of the FAM19A4/miR124-2 methylation test will be validated in different, well characterised clinical cohorts from different European countries.

Objective - Determine the inter-laboratory agreement of the FAM19A4/miR124-2 methylation test on HPV-positive cervical scrapes collected in different European screening cohorts.

Methods

In total 695 HPV-positive cervical scrapes from five different European screening settings were included, i.e. Slovenia (SL, n=97), Spain (SP, n=239), Scotland (SC, n=96), Germany (G, n=159), and Denmark (D, n=104). Cervical scrapes had been collected in PreservCyt (SP, SL, SC, and G) or Surepath (D) and DNA extraction was performed according to local procedures. The samples were tested locally and sent to the reference lab for retesting. Both test and reference lab performed separately bisulfite- conversion followed by FAM19A4/miR124-2 methylation test (QIAsure Methylation Test) according to manufacturers’ instructions. Testing at both sites was performed blinded to the results of the other lab, and compared afterwards.

Results
Overall inter-laboratory agreement was 90.5% (629/695; 95%CI:88-92) with even higher agreement values in women with CIN3+ (i.e. 96.7%; 29/30; 95%CI:80-100). Agreement values for the five screening settings ranged from 83.9% to 96.8%. Overall kappa value was 0.77 (range laboratories: 0.64-0.91) indicating good inter-laboratory agreement. Discordant test results related to samples of women without clinical relevant disease having methylation values around the clinical cut-off of the assay.

Conclusion

The inter-laboratory agreement of the FAM19A4/miR124-2 methylation test (QIA sure Methylation Test) was consistently high for the different European screening settings. Thus, the FAM19A4/miR124-2 methylation test is a good and reliable molecular triage assay suited for full molecular screening (i.e. HPV-DNA testing in combination with QIA sure Methylation Test).

References

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FC 12-02
THE SCOTTISH HPV ARCHIVE - A RESOURCE FOR BASIC AND TRANSLATIONAL RESEARCH

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Background / Objectives

Continuous research is crucial to improve our understanding for better management of HPV associated diseases. Avenues of research include mechanistic studies of HPV infection, lifecycle and pathogenesis; innovation in HPV detection technologies; biomarker discovery; development of prophylactic agents and better treatment technologies. A population based sample archive, with well-annotated and quality controlled clinical materials, assists in such research.

Methods

The Scottish HPV Archive1, setup in 2009, is a biorepository of cervical samples from women in Scotland. The archive received government core-funding for the first 5 years and then has been sustained via research funding and a revenue model based on sample provision. At the outset, archival and data management procedures along with an integrated inventory system were established. Generic Research Tissue Bank approval was obtained for sample storage and for data linkage to national databases for cervical screening, immunisation, colposcopy and cancer.

As a dynamic archive, the samples constitute residual material from different collections and include samples from women attending routine screening in addition to research collections associated with specific inclusion criteria. Current collection contains over 40,000 samples, which include 34,321 liquid based cytology, 7,913 DNA and 913 self-taken vaginal swabs. Samples are annotated with HPV infection results and genotypes, cytology and histology results and vaccination status. Quality assessment is performed regularly to assess best storage conditions for viable cells, DNA, RNA and protein. Access to samples is obtained through application to the archive steering committee2.

Results

The archive has been associated with much activity and output; to date, 37 applications have been approved for use of samples and/or data with ~14,000 samples provided. The requests are associated with research into HPV epidemiology
(4, 10.8%), biomarker development (23, 62.2%), validation and assessment of HPV detection assays (9, 24.3%), and data linkage studies (1, 2.7%). The requests have been both from United Kingdom (31, 83.9%) and international partners (6, 16.2%); and 13 (35.1%) involved commercial collaborations. The archive has contributed to 19 peer reviewed publications, 57 international conference submissions and has been a part of 14 equitable grant awards since its setup.

Conclusion

In the eight years since its establishment, the Scottish HPV Archive has proved to be a valuable resource for researchers. Our aim is to continue to engage with scientific and clinical community to ensure the archive can adapt to reflect and accommodate key and contemporary research priorities.

References

1 www.shine.mvm.ed.ac.uk/archive

2 hparchive@ed.ac.uk
FC 12-03
METHYLATION BIOMARKERS TO TRIAGE HPV POSITIVE SUREPATH COLLECTED SCREENING SAMPLES

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Background / Objectives

Implementation of primary human papillomavirus (HPV) screening will require triage of high-risk (hrHPV)-positive women to efficiently identify those with high risk of cervical high-grade intraepithelial neoplasia (CIN) and cancer, but equally importantly, to deselect hrHPV-positive women who are at low risk. Here, we evaluate the QiaSure methylation assay measuring the human biomarkers FAM19A4 and mir124-2 in combination.

Methods

Post-cytology residual SurePath samples from a group of 502 hrHPV positive women undergoing routine cytology screening at Hvidovre Hospital, Denmark, were collected (age 30-65, average: 49 years). HPV testing was done using Onclarity HPV test (BD Diagnostics, Sparks, MD) or CLART2 HPV array (Genomica, Madrid). Women with cytology abnormalities and/or hrHPV positive were referred to follow-up in concordance with Danish Guidelines. In total, 361 of the 502 women had histology registered in the Danish Pathology Databank within 12 months after the positive screening sample. Samples were reflex tested using the QiaSure methylation assay (Qiagen, Hilden, Germany). All molecular testing was performed in concordance with manufacturer’s specification. Clinical performance estimates of reflex methylation for the detection of ≥CIN3 were determined.

Results

Among the 361 women with histology, 8 had CxCa, 54 CIN1, 24 CIN2, 61 CIN3, 214 were normal and 1 had inadequate histology. For ≥CIN3, hrHPV/methylation analysis showed 77% sensitivity, PPV of 40% and NPV of 93%.

Conclusion

In a primary screening setting for women ≥30 years of age, where referral for colposcopy directed biopsies is defined by hrHPV status, the use of QiaSure methylation assay works with SurePath collected cervical samples. The resulting sensitivity, PPV and NPV support that the QiaSure methylation assay can be
considered as part of a unified molecular work flow for future molecular cervical screening, saving laboratories the work load of reflex cytology on hrHPV positive screening samples.

References

This work was supported by the SME Instrument in the Horizon 2020 Work Programme of the European Commission (Valid-screen 666800).
METHYLATION PATTERN SWITCH BETWEEN LOW AND HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA: IMPLICATIONS FOR PROGRESSION MODELS, ROBUST TRIAGE, AND CANCER RISK

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Background / Objectives

Human papillomavirus (HPV) infection accounts for an estimated 530,000 cervical cancer cases and 270,000 deaths annually, with the majority (86% of cases and 88% of deaths) occurring in developing countries. Infection with high-risk HPV types can lead to mild, moderate or severe cervical intraepithelial neoplasia (CIN1, 2 or 3) with a small percentage of persistent HPV infections and CIN3 subsequently developing into cancer. Study of viral persistence and molecular pathways in precancerous lesions is critical for an understanding of cervical carcinogenesis and optimal prevention strategies.

We assessed DNA methylation levels of host and HPV genes within the microenvironment of individual discrete lesions to look for significant associations between HPV genotype, methylation, and lesion severity in cervical surgical tissues and corresponding exfoliated cell specimens.

Methods

354 CIN were macrodissected from surgical specimens provided by 127 women who underwent loop electrosurgical excision procedures (LEEP). Samples were HPV genotyped and DNA methylation of EPB41L3 and viral regions of HPV16L1 and L2, HPV18L2, HPV31L1 and HPV33L2 were measured by quantitative pyrosequencing of bisulfite converted DNA1,2,3.

Results

Adjacent CIN of different grades usually contained the same hrHPV types. However, methylation patterns differed significantly and were much more characteristic of histopathological grade. Methylation levels in all CIN1 were the same regardless of whether a CIN1 was adjacent to CIN3 or was the highest diagnosis on the cervix. There was a significant trend of increased methylation with disease progression from normal and CIN1 to CIN3 (p<0.0001). A popular notion is that cervical carcinogenesis is a continuous progression from CIN1, CIN2, and finally CIN3 to cancer. However, experimental evidence indicates that CIN1 is not necessarily a direct precursor of
CIN3, instead different grade lesions can develop directly in a simultaneous or staggered timeframe. Our results indicate that elevated methylation characteristic of CIN3 seems to be related to a discrete molecular “high methylation” switch from the normal state rather than a gradual secular increase. HPV genotype and methylation results in LEEP biopsies and corresponding exfoliated cells were similar. 99% of CIN3 and 88% of CIN1 had the same or matching HPV types. There was a significant correlation between LEEP and cervical scrapes for both EPB41L3 (Spearman \( r=0.2033, \ p=0.0253, \ n=121 \)) and HPV (Spearman \( r=0.2156, \ p=0.0237, \ n=110 \)).

**Conclusion**

Our study supports the use of DNA methylation testing as a prognostic biomarker of CIN3 and cancer risk and it may be used as a robust triage for hrHPV positive women.

**References**

Diagnostic value of methylation markers in cervical cancer screening

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Background / Objectives

DNA methylation analysis has been assessed as a potential biomarker for early cervical cancer detection. In this review, we summarize the studies analyzing the diagnostic potential of methylation markers in cervical scrapings by Quantitative Methylation Specific PCR (QMSP).

Methods

All studies, until February 1 2017, were systematically searched from three electronic databases (Pubmed/Medline, Embase and Cochrane). Studies on cervical scrapings that used (Q)MSP for methylation analysis and histology as the golden standard were retrieved. Sensitivity and specificity of methylation markers were extracted to assess the diagnostic values of methylation markers. Data were stratified for studies using methylation markers analysis as primary test versus those as triage test after primary HPV screening.

Results

In total 699 studies were retrieved, of which 68 studies describing 89 genes fulfilled our criteria. Preliminary analysis revealed 21 methylation markers as primary test comparing normal/low-grade squamous intraepithelial lesions (LSIL) versus high-grade (H)SIL and cancer. Six genes (including EPB41L3 and JAM3) were identified with relatively high sensitivity (49%-100%) and specificity (67%-100%) to detect HSIL. Eight genes (including EPB41L3 and JAM3) showed high sensitivity (70%-100%) and specificity (78%-100%) for detecting cancer. Methylation analysis as triage test in HPV positive women resulted in 19 genes, of which 11 genes (including EPB41L3 and JAM3) showed combined high sensitivity (53%-100%) and specificity (71%-100%) for HSIL or worse, which was comparable or higher than other triage strategies.

Conclusion
The preliminary results of this review reveal that multiple methylation markers have been analyzed in either primary or triage test. Especially, triaging hrHPV positive women by methylation analysis is interesting for implementation in population-based screening where sensitivity can be even improved by combining markers without losing specificity. However, to confirm the relevance of selected methylation markers, further validation needs to be performed in large population-based settings.
FC 12-06
FAM19A4/MIR124-2 METHYLATION ANALYSIS FOR CERVICAL CANCER SCREENING IN WOMEN LIVING WITH HIV

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Background / Objectives

Women living with HIV (WLHIV) are at increased risk for developing cervical intraepithelial neoplasia (CIN) and cervical cancer. Countries with a high HIV incidence, such as South Africa, often lack effective cervical cancer screening programs due to insufficient resources, lack of infrastructure and limited access to health care. Reliable tests for early cervical cancer and precancer detection that are suitable for this setting are urgently needed.

This study evaluates the performance of FAM19A4/miR124-2 methylation markers (QIASure Methylation Test) on physician-taken cervical scrapes to detect cervical cancer and CIN grade 3 (CIN3) in WLHIV in South Africa.

Methods

Samples from a prospective observational multi-centre cohort study were used for this analysis. In this study, two cohorts were included: a cohort of WLHIV who were invited for cervical cancer screening (n=321) and a referral cohort consisting of women referred for further evaluation of a cervical abnormality in a gynaecologic outpatient department (n=108). Cervical scrapes collected from all subjects were used for methylation analysis of FAM19A4 and miR124-2 genes by the QIASure Methylation Test. High-risk HPV (hrHPV) status and histology endpoints were available for all subjects. Methylation levels in samples of HIV seropositive women were compared to samples of HIV seronegative women. Performance of reflex methylation analysis among hrHPV-positive women for detection of CIN3 or worse (CIN3+) was determined in the cohort of WLHIV.

Results

Methylation levels increased with severity of cervical disease in both study cohorts and were above the set threshold in all samples of women with cervical cancer. When compared to samples of HIV seronegative women, methylation levels in samples of WLHIV were significantly higher in all histology groups except cervical cancer. Stratifying hrHPV-positive women with reflex methylation analysis showed a CIN3+ sensitivity of 72.9% and a specificity of 76.1%.
Conclusion

In this South African cohort of WLHIV, reflex methylation analysis of hrHPV-positive cervical scrapes detects all cervical carcinomas and has an acceptable sensitivity and specificity for CIN3+ detection. The applicability of the test on self-collected samples and its objective nature makes it a promising screening tool for low-resource settings.
FC 12-07
HPV DNA METHYLATION AS A BIOMARKER FOR IMPROVING RISK STRATIFICATION AND CLINICAL MANAGEMENT OF HPV-POSITIVE WOMEN

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Background / Objectives

While HPV DNA testing has greater sensitivity compared to cytology, specificity is lacking, and triage tests are required to distinguish benign HPV infections from precancers. A promising triage option is HPV DNA methylation (DNAm). Increased HPV DNAm has been associated with precancer in four major carcinogenic types (HPV16, 18, 31, 45). We hypothesize that DNAm is an important step in carcinogenesis common to all HPV types. To test this hypothesis, we conducted a nested case-control study evaluating the association of HPV DNAm with cervical precancer for 12 carcinogenic HPV types.

Methods

For 12 HPV types, we selected 30 cases of cervical intraepithelial neoplasia grade 3 (CIN3) and 30 controls without abnormalities from a population of HPV-positive women. HPV DNAm in viral L1 and L2 genes (about 9 CpG sites per type) was measured using next-generation bisulfite sequencing. We calculated odds ratios (OR) using logistic regression for the association of DNAm with precancer of DNAm and assessed the possible discrimination between infection and precancer using areas under the curve (AUC). For each HPV type, we calculated specificity at a fixed sensitivity of 85% and weighted back to all HPV-positive women to estimate the risk in methylation positive and methylation negative subjects. These estimates were compared with established management thresholds (colposcopy referral).

Results

We observed significant associations of higher DNAm with precancer in all but 3 sites (OR range 4-28.0). For each HPV type, the highest AUCs were 0.91 (HPV59), 0.86 (HPV18), 0.85 (HPV39), 0.84 (HPV16), 0.82 (HPV45), 0.81 (HPV35), 0.77 (HPV52), 0.74 (HPV58), 0.75 (HPV31), 0.73 (HPV33) and 0.71 (HPV56 and HPV51). At fixed Se of 85%, the Sp for DNAm across HPV types was similar to that of cytology, and ranged from 26.7% (HPV51) to 90.0% (HPV59). Weighting back to all HPV-positive women, the risk of CIN3+ in methylation–positive women was clearly above the colposcopy referral threshold.

Conclusion
We observed a strong association of increased HPV DNAm with precancers across 12 HPV types, suggesting that DNAm is a general phenomenon in the transition from infection to precancer. For most types, clinical performance of DNAm was comparable to or exceeded that of cytology. Next, we will analyze a combined panel of DNAm sites from each HPV type in a large screening population. We plan to develop an assay that provides risk stratifying information based on HPV genotyping and DNAm for the clinical management of HPV-positive women, which can be measured in a variety of specimen types, including self-collected samples, which are not amenable for cytology.
CLINICAL VALIDATION OF POU4F3 METHYLATION AS A NEW BIOMARKER OF CERVICAL PRECANCER AND CANCER IN A TRIAGE OF HRHPV POSITIVE WOMEN

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Background / Objectives

The ongoing TRACE prospective, multicenter study provided a thorough clinical evaluation of the POU4F3 methylation by using the CONFIDENCE Marker™ RUO test in HPV triage by comparison to cytology triage. A new version of POU4F3 methylation test, called CONFIDENCE Marker™ (IVD-CE) was developed with the intended use of triaging hrHPV positive women aged 30 years or older and giving an indication about the women's current CIN2+ risk.

Methods

CONFIDENCE Marker™ (IVD CE) measures the relative methylation level of the promoter region of the gene called POU4F3 by quantitative methylation specific real-time PCR (qMSP) technology compared to the reference gene COL2A1 (providing a so-called Methylation index). Clinical performance of the CONFIDENCE Marker™ (IVD CE) was assessed on hrHPV positive (CONFIDENCE HPV™) LBC samples (CIN2- n=187; CIN2+ n=26) selected from the TRACE study collected from subjects aged 30 years or older. The results of the CONFIDENCE Marker™ (IVD CE) for CIN2+ and CIN3+ clinical endpoints and their agreement with the CONFIDENCE Marker™ RUO test results in the TRACE study was calculated.

Results

The CONFIDENCE Marker™ (IVD CE) achieved sensitivity of 88.5% (69.8-97.6%) with the relative sensitivity of 0.96 (0.77-1.19) and specificity of 69.6% (47.1-86.8%) with the relative specificity of 1.13 (0.79-1.59) for the histologically confirmed samples and 88.5% (69.8-97.6%) with a relative sensitivity of 0.96 (0.77-1.19) and 75.9% (69.2-81.9%) with a relative specificity of 0.99 (0.88-1.11) calculated on all samples, respectively for CIN2+ histological endpoint in the age group 30-65 of hrHPV positive women. The relative values were assessed by comparison to the CONFIDENCE Marker™ RUO test results in the TRACE study. The overall agreement of the two CONFIDENCE Marker™ test workflow results was 96.2% (80.4-99.9%) and 100% for CIN2+ and CIN3+ endpoints, respectively. The current analysis is focused on the baseline cross-sectional clinical results, the 3 years follow-up of the study is ongoing.

Conclusion
Based on the relative sensitivity and specificity values obtained, TRACE’s clinical evaluation of the CONFIDENCE Marker™ RUO can be considered valid for the CONFIDENCE Marker™ (IVD CE) as the results do not show a significant statistical difference.

On the basis of our findings, one of the first IVD-CE validated methylation assay, the new CONFIDENCE Marker™ (IVD CE) detecting the POU4F3 methylation as a triage test of hrHPV positives appears to be a promising method. We can reasonably assume that its quantitative nature offers the potential of an objective and discriminative risk assessment tool in the prevention and diagnostics of high-grade CIN lesions and cervical cancer.
ASSOCIATIONS OF EPB41L3 DNA METHYLATION WITH CERVICAL INTRAEPITHELIAL NEOPLASIA IN WOMEN LIVING WITH HIV-1 IN BURKINA FASO AND SOUTH AFRICA

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Background / Objectives

To evaluate the association of DNA methylation of the human gene EPB41L3 with high-grade cervical intraepithelial neoplasia (CIN2+) and HIV-related factors among women living with HIV-1 (WLHIV) in Burkina Faso (BF) and South Africa (SA).

Methods

Case-control study of WLHIV aged 25-50 with histology-determined CIN2+ (cases, N=152) or without lesions (≤CIN1, controls, N=210). Methylation levels of EPB41L3 were measured by pyrosequencing of exfoliated cervical specimens. Among 185 controls that were followed over a median 16 months (endline), methylation levels were measured among 26 incident CIN2/3 and 159 controls that remained ≤CIN1 at endline. Methylation levels were dichotomized using the 66.7 percentile among controls in each country for high/low cut-off.

Results

The median methylation levels for EBP41L3 were significantly higher among women with prevalent CIN2/3 compared to those with ≤CIN1 in both countries (Cuzick p for trend by CIN grade <0.001). Women with CD4+ count ≤200 cells/mm3 were more likely to have higher levels of EPB41L3 methylation compared to women with CD4+ >350 cells/mm3 at baseline (BF: adjusted Odds Ratio [aOR]=7.45, 95%CI 1.53-36.22; SA: aOR=2.74, 95%CI 1.16-6.47; adjusted for HR-HPV and CIN status). Among 36 women with prevalent CIN2+ at baseline who had not gone for treatment by endline visit in SA, women with persistent CIN3, or CIN2 which progressed to CIN3 had higher baseline EPB41L3 median methylation levels
compared to women who spontaneously regressed to ≤CIN1 (Mann-Whitney p=0.016).

**Conclusion**

Methylation of human gene EPB41L3 DNA is elevated in prevalent CIN2/3, and incident and persistent CIN3 cases, and independently associated with lower CD4 count. DNA methylation based assays could be useful in settings with limited resources for management, by identifying women most likely to have CIN3 that will persist or progress.
Cervical cancer detection by DNA methylation analysis in urine


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Background / Objectives

Cervical screening programs using cervical cytology, are or will be replaced by primary hrHPV testing in several countries. Women who test hrHPV-positive require a secondary (triage) test to prevent over-referral and over-treatment. Analysis of DNA methylation of host cell tumor suppressor genes in cervical scrapes provides promising triage strategy for hrHPV-positive women. Urine collection is expected to increase the uptake of cervical screening programs, and hrHPV testing in urine appears promising. We aimed to test whether DNA methylation analysis in urine provides a novel more patient-friendly strategy to detect cervical cancer.

Methods

Cervical scrapes and urine samples from cervical cancer patients and healthy female controls (n=40 each) were tested for hrHPV DNA presence and DNA methylation of 6 genes with known performance of cervical (pre)cancer detection in cervical scrapes.

Results

A high concordance was found between hrHPV DNA testing on cervical scrapes and urine samples. Also DNA methylation levels in paired cervical scrapes and urine samples showed a good correlation. DNA methylation levels of all 6 genes were significantly increased in urine samples of cervical cancer patients compared to controls. Receiver operating characteristics (ROC) analysis of the 6 methylation markers in urine showed AUCs ranging from 0.88 to 0.95.

Conclusion

DNA methylation testing in urine is feasible and has a high accuracy to detect cervical cancer. These data warrant further exploration of methylation markers in urine-based cervical screening programs.
FC 12-11
GYNTECT®, A DNA METHYLATION MARKER PANEL-BASED DIAGNOSTIC TEST SHOWS VERY HIGH SPECIFICITY IN THE TRIAGE OF CERVICAL CANCER SCREENING SAMPLES

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Background / Objectives

A change of the current screening algorithms to a HPV-based screening setting is discussed in several countries due to higher sensitivity of HPV testing compared to cytology. Reliable triage methods are, however, essential in such a setting to avoid overtreatment and higher screening costs. Specific DNA methylation patterns may provide a suitable tool especially with regard to keeping false positive rates low.

Methods

Cervical scrapes collected in PreservCyt® from women with cervical cancer (5 cases), CIN 1-3 (74 cases) and normal cytology (Pap I; 200 cases) were assessed for methylation of the marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671 (GynTect® assay). All samples had previously been tested for HPV by the cobas® HPV assay. Moreover, for all patients with CIN or cancer and for 59 of 200 patients with Pap I data for p16/Ki67 dual staining (CINtec Plus® test) were available.

Results

All samples from women with cervical cancer, 61.2% of CIN3, 44.4% of CIN2 and 20.0% of CIN1 cases were scored positive for the GynTect methylation assay. The specificity within the Pap I group was 98.5%, thus showing an exceptionally low false-positive rate. Overall, the number of methylated marker regions increased proportionally to lesion severity, which is in contrast to CINtec Plus® and cobas® HPV, of which both detect all CINs irrespective of severity grade. Specificity of CINtec Plus in the Pap I group was similar, even though the tested cohort was smaller. We plan to have CINtec Plus results for all 200 Pap I samples before the Eurogin conference. Specificity of the cobas HPV in the Pap I group was 92%.

Conclusion

DNA methylation analysis of the above marker panel (GynTect® test) in cervical scrapes consistently detects cervical cancer and the majority of CIN3 as well as a subset of CIN1/2 lesions, whereas the positivity rate among cytology-normal samples is extraordinarily low. Altogether, the GynTect® assay based on detection of six
methylation markers may provide an excellent tool for triage within cervical cancer screening.
FC 12-12
BETA-GLOBIN CYCLE THRESHOLD VALUE AS A PREDICTOR OF SUFFICIENT DNA YIELD FOR HPV METHYLATION ANALYSIS

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Background / Objectives
Since several countries adopted HPV testing as a primary screening method for cervical cancer, different triage strategies for high-risk HPV positive women are being evaluated in order to avoid misinterpretation and mismanagement of patients. Reflex cytology, partial HPV genotyping and host and viral methylation are the most commonly evaluated strategies to date. The aim of our study was to assess whether there is a correlation between the beta-globin cycle threshold (Ct) value and the concentration of extracted DNA in order to predict sufficient yield of DNA for methylation.

Methods
For the purpose of this study, we have evaluated 195 samples that were tested with clinically validated HPV test Abbott RealTime High Risk HPV test (RealTime; Abbott, Wiesbaden, Germany) at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia between January 2016 and March 2016. The internal control of the RealTime is based on amplification of the 136-bp region of the beta-globin gene. Furthermore, DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and DNA concentration was measured with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA). Samples with DNA concentration above 2.5 ng/ul were considered eligible for further HPV methylation analysis.

Results
Ct values for beta-globin ranged between 20.26 to 31.25, with an average Ct value of 24.57. Of the 195 samples tested, 92 had DNA concentration above 2.5 ng/ul, 98 samples below 2.5 ng/ul and 5 below measurable concentration (range: 0.144-40,0 ng/ul). Our analysis showed that 93.8% of the samples with Ct values below 22.00 (15/16), 87.7% of the samples with Ct values between 22.00 and 24.00 (57/65) and 29.0% of the samples with Ct values between 24.00 and 26.00 (18/62) had sufficient DNA yield for methylation, respectively. Additionally, only two out of 52 samples with the Ct value above 26.00 had a concentration above the cut-off value (one sample with Ct value 26.15 had DNA concentration 3.42 ng/ul and one sample with Ct value 26.28 had DNA concentration 4.28 ng/ul).

Conclusion
In HPV primary screening settings a well-balanced triage strategy will be needed to identify women with higher risk of underlying cervical intraepithelial neoplasia grade 2 or worse. Our results suggest that Ct values of beta-globin of clinically validated HPV test RealTime can be used as a preliminary indicator for sufficient DNA concentration needed for methylation analysis.
LONGITUDINAL PERFORMANCE OF HPV 16 METHYLATION PREDICTING CERVICAL PRECANCER AND CANCER: A 10-YEAR COHORT STUDY IN CHINA


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Background / Objectives

To evaluate the predictive ability of methylation of the most common human papillomavirus(HPV16) L1 region and Long Control Region(LCR) for cervical precancer and cancer. And to assess the correlation of methylation between different CpG sites, as well as between methylation and HPV viral load.

Methods

A hospital-based case-control study consisting 27 HPV16 single positive women was designed to identify the significant CpG sites. Then 10-year population-based screening cohort conducted in cervical cancer high-incidence area (Shanxi province) was used to valid the identified CpG sites. The cohort included 1742 women followed up in 2005, 2010 and 2014 using HPV testing and cytology. Women with any positive screening result received colposcopic examination and biopsy if necessary. Based on the combination of biopsy result at 3 follow-up visits, women were classified into CIN2+ persistence/progression group and regression group to assess the longitudinal performance of previously identified CpG sites. DNA extracted from cervical cytology specimens was quantified for methylation levels at 35 CpG sites throughout HPV16 L1 and LCR. The Mann-Whitney U test was used to compare the methylation pattern between different biopsy results. And spearman-test was used to assess the relation between different CpG sites and between HPV viral load and methylation.

Results

HPV 16 methylation increased with the grade of cervical precancer(p<0.001). The median methylation level for CIN1/normal, CIN2, CIN3+ was 11.16%, 11.54% and 23.19%, respectively. Specially, methylation of 14 CpG sites (L1:5602、5608、5611、5617、5709、5726、6367、6389、6457、6650、7034、LCR:7455、7535、7553) was significant higher among women with CIN3+ than <CIN3. Considering literature review, another 10 CpG sites were also evaluated during follow-up. 77 women diagnosed with CIN2+ were HPV16 positive in 2005. After 5 years, the methylation of 6650 was significantly higher among CIN2+ persistence/progression group, another CpG(nucleotide position 31) also showed the predictive ability for CIN2+ after 10 years. Correlation was found between many different CpG sites, especially, the correlation coefficient between 6367, 6389 and 52 were both more...
than 0.7. No strong relationship was identified between HPV viral load and methylation level stratified by biopsy result. The max correlation coefficient was no more than 0.4.

**Conclusion**

HPV 16 methylation of L1 and LCR can be used as biomarker for cervical precancer and cancer. The methylation of 6650 in L1 showed promising predictive ability for the progression of cervical precancer. The correlation was identified between different CpG sites, but HPV viral load was not related to methylation level.
DEVELOPMENT OF A NEW HIGHLY ACCURATE DNA METHYLATION CLASSIFIER FOR PREVALENT AND INCIDENT CERVICAL PRECANCER

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Background / Objectives

Cervical cancer, caused by persistent infection with high risk (hr) HPV affects ~500,000 women globally and causes ~260,000 deaths annually. Although HPV immunisation has been successfully implemented it will take decades to see an effect of the new nonavalent vaccine. Highly sensitive hrHPV testing is likely to become the dominant primary screen. However, hrHPV infection is common and only a fraction of women are at risk of developing cancer. 40% of hrHPV+ women are cytology negative and triage by proposed adjunctive tests such as p16 (in conjunction with ki67) are insufficient. A molecular test is needed to identify clinically significant HPV infection.

We aim to identify and validate novel DNA methylation biomarkers which, in combination with our existing DNA methylation classifier¹²³, will aid development of a new improved classifier for identification of high grade CIN and ultimately improve the current cervical cancer screening gold standard. To achieve this, we propose to measure DNA methylation status of predefined sites in HPV16, 18, 31 and 33 in a set of 350 hrHPV+ women with normal cytology. In addition we will measure genome scale human DNA methylation with RRBS (Reduced representation bisulfide sequencing) followed by machine learning using MS-SPCA to identify ~100 sites which consistently appear in the best ranking models. Then, the best sites will be further sifted by additional multivariate data modelling to provide us with a minimum number of required classifier sites. Finally, these selected sites will be validated in a second set of 200 well characterised cervical samples.

Methods

DNA was purified from frozen LBC samples for methylation testing using Qiagen DNA extraction kits following standardized methods¹²³. The RRBS method was designed to obtain 30 million reads per sample using 50bp SE reads corresponding to 20X depth coverage of each CpG. All specimens used in the project from the ARTISTIC and Scottish HPV Archive will be tested for HPV DNA methylation by pyrosequencing³. The HPV sites tested are selected according to established knowledge about their role and importance⁴.

Results
The preliminary results indicate that this approach significantly improved risk classification tool to triage hrHPV+ women to colposcopy. The new classifier is expected to come close to the high sensitivity of current hrHPV tests (90-95%) but deliver a substantially higher specificity and PPV (both ~70%) than current molecular reflex tests (30-40% and 40-50% respectively).

Conclusion

This new algorithm would allow more efficient utilization of colposcopy services while hrHPV+ women negative for the triage classifier could be followed up at suitably frequent intervals to safely catch most, if not all, triage false negatives.

References

RISK ALLELIC LOAD IN TH2 AND TH3 CYTOKINES GENES AS BIOMARKER OF SUSCEPTIBILITY TO HPV-16 POSITIVE CERVICAL CANCER: A CASE CONTROL STUDY

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Background / Objectives

Background: Alterations in the host cellular immune response allow persistent infections with High-Risk Human Papillomavirus (HR-HPV) and development of premalignant cervical lesions and cervical cancer (CC). Variations of immunosuppressive cytokine levels in cervix are associated with the natural history of CC. Objectives: To assess the potential role of genetic host immunity and cytokines serum levels in the risk of developing CC, we conducted a case–control study paired by age.

Methods

Methods: Peripheral blood samples from patients with CC (n = 200) and hospital controls (n = 200), were used to evaluate nine biallelic SNPs of six cytokine genes of the adaptive immune system by allelic discrimination and cytokines serum levels by ELISA.

Results

Results: After analyzing the SNP association by multivariate logistic regression adjusted by age, CC history and smoking history, three Th2 cytokines (IL-4, IL-6 and IL-10) and one Th3 (TGFB1) cytokine were significantly associated with CC. Individuals with at least one copy of the following risk alleles: T of SNP (−590C > T IL-4), C of SNP (−573G > C IL-6), A of SNP (−592C > A IL-10), T of SNP (−819C > T IL-10) and T of SNP (−509C >T TGFB1), had an adjusted odds ratio (OR) of 2.08 (95 % CI 1.475–2.934, p = 0.0001), an OR of 1.70 (95 % CI 1.208–2.404, p = 0.002), an OR of 1.87 (95 % CI 1.332–2.630, p = 0.0001), an OR of 1.67 (95 % CI 1.192–2.353,
p = 0.003) and an OR of 1.91 (95 % CI 1.354–2.701, p = 0.0001), respectively, for CC. The burden of carrying two or more of these risk alleles was found to have an additive effect on the risk of CC (p trend = 0.0001). Finally, the serum levels of Th2 and Th3 cytokines were higher in CC cases than the controls; whereas IFNG levels, a Th1 cytokine, were higher in controls than CC cases.

Conclusion

Conclusion: The significant associations of five SNPs with CC indicate that these polymorphisms are potential candidates for predicting the risk of development of CC, representing a risk allelic load for CC and can be used as a biomarker of susceptibility to this disease1.

References

COMPRESSED COMPARTMENTAL MULTI-TYPE HPV MODELS – CAN THEY BE USED TO INFORM CERVICAL CANCER SCREENING?

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Background / Objectives

Numerous options exist for cervical cancer prevention with new screening and vaccination modalities. Microsimulation models are used to investigate these options and predict the cost-effectiveness of integrated strategies but are computationally intensive. Besides, because the outcomes of micro-simulation models are not available in closed form, the execution of probabilistic sensitivity analyses, or parameter estimation, by Bayesian procedures remains challenging. Compartmental models may also be considered. They are fast and provide numerical closed form solutions, but the number of compartments rapidly increases with the number of HPV types and complexity of the screening algorithm.

Methods

We developed a compartmental mathematical progression model that integrates natural history of cervical carcinogenesis along multiple HPV types and screening interventions. The full model was compressed to achieve executable and efficient model, even when dealing with complex screening. The model outcomes are available in closed form so that the parameter uncertainty can be assessed by Bayesian procedures. The models were compared to a microsimulation approach in terms of overall and type-specific HPV prevalence of intermediate stages, screening outcomes and cancer incidence.

Results

The outcomes of the compressed compartmental model were stable over different levels of compression and stayed between the simulation error bounds of the microsimulation model for all overall HPV infection states. A small difference was observed in a fraction susceptible versus immune.

Conclusion

Good approximation properties of compressed compartmental models enable us to assess uncertainties surrounding the natural history of cervical carcinogenesis and screening decisions in a computationally undemanding way.
The cost-effectiveness of national HPV immunization programmes in six European tender-based settings

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Background / Objectives

Vaccination of preadolescent girls against HPV 16/18 within national immunization programmes has been established in most European countries, but coverage varies substantially between countries. In addition, HPV vaccines in many countries are purchased via national public procurements that reduce the cost per vaccinee considerably. This study sought to evaluate the cost-effectiveness profile of gender-neutral national HPV immunization programmes in settings with organized tender procedures for the acquisition of the HPV vaccine.

Methods

A previously published Bayesian synthesis framework was expanded to account for the full spectrum of the HPV-related cancers in both males and females and for all herd immunity effects from vaccinating girls as well as boys. Our analysis assessed the cost-effectiveness of a sex-neutral vaccination programme within 6 European countries (Austria, Belgium, Croatia, Latvia, the Netherlands and Sweden) for which we collected publicly available information on national tender procedures. Country and site-specific incidence data from the last edition of the Cancer Incidence in Five Continents (CI5) were used to inform the model and national mortality data were obtained from the World Health Organization mortality database.

Results

The incremental cost-effectiveness ratios (ICERs) of girls-only vaccination compared to no vaccination ranged from €500 (95% CrI: 0 - 1,000) per life-year gained in Latvia to €5,000 (95% CrI: 4,000 - 6,000) per life-year gained in Austria, while the incremental cost-effectiveness ratios of the sex-neutral vaccination programmes compared to girls-only vaccination ranged from €4,000 (95% CrI: 3,000 - 6,000) per life-year gained in Croatia to €26,000 (95% CrI: 20,000 - 33,000) per life-year gained in Sweden. The ICERs remained below the country-specific GDP thresholds for cost-effective intervention, recommended by the WHO. Ninety-five percent of the variation in ICERs for sex-neutral vaccination among countries could be explained by coverage among girls, vaccination cost, cervical cancer incidence and survival, and oropharyngeal cancer incidence in males.

Conclusion
Gender-neutral vaccination against HPV is likely to be cost-effective in settings where tender procedures can be organized for the acquisition of the HPV vaccine in national immunization programmes. This finding seems generalizable over a wide range of epidemiologic and economic constraints.
Cost-effectiveness of expanding the HPV vaccination program to include preadolescent boys in Sweden

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Background / Objectives

Since 2012, vaccinating against human papillomavirus (HPV) in preadolescent girls in schools has been a part of the national vaccination programme for children in Sweden. HPV vaccination coverage among girls has since been around 80%. The main goal with the introduction of the vaccine was to protect girls from infection with high-risk types of HPV that may cause cervical cancer. The vaccine also prevents other cancer types of which some are prevalent among men, such as oropharyngeal, anal, and penile cancer. The aim of this study was to assess the cost-effectiveness of including HPV-vaccination for preadolescent boys in the Swedish national immunization program by comparing health effects and costs of all HPV-related disease in a situation with a gender neutral vaccination programme compared to only vaccinating girls.

Methods

We used a dynamic compartmental model to simulate the transmission of HPV 16/18 in the population, accounting for indirect effects of vaccination through herd immunity. The model accounted for sexual behaviour, such as age preferences of sexual contacts and men who have sex with men. The main outcome was number of individuals with all HPV-related cancers as well as CIN. The data in the model were based on epidemiological studies, demographic statistics, cancer registers and other Swedish population-based healthcare and sociodemographic registers that capture all healthcare interactions. Estimates were calibrated to fit Swedish empirical data.

Costs included in the analysis were those incurred when treating HPV-related cancer and CIN, production losses during sick-leave, and acquisition and administration of the vaccine. Health effects were measured as quality-adjusted life years (QALY). The time horizon was set at hundred years, and both effects and costs were discounted with 3% annually. All health effects and costs were accumulated over the time horizon and used to create the incremental cost-effectiveness ratio (ICER). Several variables, such as price of the vaccine, vaccination coverage, vaccine effectiveness, and herd immunity were varied in sensitivity analyses to illustrate their impact on the results from the cost-effectiveness analysis.

Conclusion
Preliminary results indicate that a gender neutral vaccination programme will reduce HPV-related cancer and CIN during the model's time horizon, both due to direct effects of the vaccine as well as indirect effects decreasing HPV prevalence in the population. The potential cost-effectiveness of a gender neutral programme is dependent on the price of the vaccine, the lower the price the more favourable it is to vaccinate boys from a societal perspective.
OPTIMAL IMPROVEMENTS TO CERVICAL CANCER PREVENTION: EXAMPLE FROM AUSTRALIA

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Background / Objectives

There are now a variety of tools for cervical cancer prevention. HPV vaccination and screening programs are in place in several settings, however coverage is often sub-optimal. Improving participation is important, but improving vaccine uptake will involve different approaches to those required to increase screening participation or adherence to recommended follow-up. To assist in prioritizing and resourcing efforts, here we explore the impact of different potential improvements to participation in cervical cancer prevention programs, and which improvements would have the greatest impact.

Methods

Using a well-established model of HPV transmission, vaccination, natural history and screening, we assessed the relative impact of several improvements to HPV vaccination and screening participation, using Australia as an example. These improvements included: i) increasing HPV vaccine coverage in females (from current 78%); ii) increasing HPV vaccine coverage in females and males (current male coverage 72%); iii) reducing the proportion of women never screened; iv) increasing screening participation at the recommended interval; v) improving attendance for follow-up by women under surveillance following a previous abnormal screening test. The impact of improvements in screening participation were assessed separately for cohorts offered vaccination and for unvaccinated cohorts.

Conclusion

The findings of this analysis will provide important information about how to prioritise efforts in increasing participation in cervical cancer prevention programs.
BACKGROUND / OBJECTIVES

To assess the public health and economic impact of a gender neutral vaccination program for 9-14 year olds with a nine-valent human papillomavirus (HPV) in Sweden.

METHODS

A previously validated transmission dynamic model was adapted and calibrated for Sweden. The natural history of cervical cancer, CIN 1-3, vaginal cancer, vulvar cancer, anal cancer, and genital warts, was simulated in the model. The current screening program for cervical cancer in Sweden was included in the model. In the model a gender neutral vaccination program (boys and girls) for 9-14 year olds with a nine-valent HPV vaccine was compared to a girls only (9-14 year old) quadrivalent HPV vaccination program. The vaccination coverage and other inputs to the model were collected from relevant local sources where available. Life-long duration of protection was assumed in the model for vaccine HPV types for both vaccines and the model time horizon was set to 100 years. Costs and QALYs were discounted by 3%. Sensitivity analyses were performed on comparisons with adherence rates and discounting.

RESULTS

The gender neutral vaccination program resulted in an added reduction of 22% in cervical cancer incidence and mortality for females, and 13% additional decrease in anal cancer incidence and 12% mortality in females and 32% decrease in incidence and mortality in males. An additional 110,148 cases genital warts in girls and 466,960 cases in boys would be prevented if a gender neutral vaccination with a nine-valent vaccine would be introduced compared to the current scenario in Sweden. Gender neutral vaccination with a nine-valent HPV vaccine as compared to the quadrivalent vaccine resulted in an additional overall 14.5% decrease in disease specific costs.

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 Sweden.
NEW EVIDENCE WITH REGARD TO TEST CHARACTERISTICS FROM A MODELLING STUDY

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Background / Objectives

The natural history of cervical cancer is only partly known, as most precancerous lesions are either treated or regress naturally prior to becoming clinical. Also, data on test characteristics is lacking because randomized controlled trials are never performed. However, microsimulation models allow us to mimic the natural history of cervical cancer. Calibrating unknown parameters in these models, such as durations of disease states, test characteristics and demographic assumptions, to observed data provides us with more insight into unobservable processes. Recently, large cohort studies have been added to this observed data providing new possibilities for analyses. In this study, we explored what could be learned from a microsimulation model about test characteristics when screening for cervical cancer.

Methods

The established MISCAN-Cervix microsimulation model was calibrated to the Dutch setting based on the latest data from the Netherlands Cancer Registry (NCR), the nationwide network and registry of histological and cytopathology in the Netherlands (PALGA) and recently published cohort studies on HPV detection rates. To identify any differences in HPV-test sensitivity by disease grade, sensitivity was calibrated for each disease grade separately (i.e. cervical intraepithelial neoplasia (CIN)1, CIN2, CIN3 and cervical cancer). To test if false-negative tests were more likely to be attributable to the same (hard to reach) lesions, we allowed for the chance of systematically missing individual lesions with cytology testing in the model.

Results

We found that the model fitted the observed data best when the sensitivity of HPV tests increases by disease grade. Especially the sensitivity for CIN1 was considerably lower than for higher CIN grades. For cytology testing, the fit of the model was best if we allowed for systematically missing about 11% of individual lesions.

Conclusion

The model was much better able to fit the observed data when adjusting HPV test sensitivity by disease grade and systematically missing a percentage of lesions at cytology testing. A possible explanation for an increasing sensitivity of HPV-tests by disease grade is that the infection persists for a longer period of time, was able to spread more, and therefore more easily detected. Systematically missing lesions at
cytology testing is probably caused by the fact that some lesions are harder to reach with the cervical smear brush or spatula, such as adenocarcinoma. These findings could have clinical implications for screening practices as the screening guidelines depend heavily on test characteristics.
HEALTH AND ECONOMIC IMPACT OF HPV TESTING COMPARED TO CYTOLOGY: WHAT IS THE OPTIMAL PRIMARY CERVICAL CANCER SCREENING STRATEGY FOR CANADA?

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Background / Objectives

To examine the incremental effectiveness and cost-effectiveness of switching from cytology-based routine screening to primary HPV testing in Quebec (Canada), assuming 9-valent HPV vaccination.

Methods

We used HPV-ADVISE, an individual-based transmission-dynamic model of 18 HPV types and related diseases calibrated to Canadian-specific data. We compared cytology-based screening vs. switching to primary HPV-testing (Cobas 4800 test & triage of HPV-positive women by cytology) in 2018. For vaccination impact predictions, we modelled Quebec’s vaccination program: vaccination coverage=80%, start of vaccination=2008, gender-neutral 9-valent vaccination. For our base-case Cytology screening scenario, we used Quebec’s proportion of women who ever had a cytology test, and current age distribution of first screening and adherence to screening intervals. For HPV testing scenarios, we varied age at start of screening and screening intervals. We used a health care provider perspective, 3% discount rate, 2018-2050 time horizon, and $40,000/QALY-gained cost-effectiveness threshold. Predictions are annual averages for a population of 10 million.

Results

In Quebec, switching to HPV screening was predicted to result in substantial cost savings vs. Cytology screening (savings of $27-38 million/year on average), under scenarios where age at start of screening was 25-35 years old, and intervals between tests were between 5-10 years. However, the only HPV screening strategy investigated with equal or lower rates of cervical cancer was when assuming age at start is 25 years and interval between tests is 5 years. Older age at start or wider intervals led to a predicted increase in cervical cancer cases. In terms of cost-
effectiveness, HPV screening every 10 years initiated at age 30 years was predicted to be the optimal scenario. Although this scenario could lead to a small increase in cervical cancers, it would also result in a substantial decrease in false positives that are referred to colposcopy. Consequently, the model predicts that the gains in Quality-Adjusted Life-Years (QALY) related to screening outcomes outweigh the QALY loss related to the increase in cancer cases.

**Conclusion**

In Quebec, switching from Cytology to HPV screening is predicted to produce substantial cost savings and important reductions in false positive rates, if there is good adherence to the recommended screening intervals. However, the only scenario predicted to decrease cervical cancer rates is 5-yearly HPV screening initiated at age 25 years. Finally, HPV-screening every 10 years initiated at age 30 is likely the most cost-effective scenario, although it could lead to a slight increase in the number cervical cancers.
HEALTH-RELATED QUALITY OF LIFE IN THE
PREVENTION, SCREENING AND MANAGEMENT OF
CERVICAL DISEASE: A SYSTEMATIC REVIEW

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Background / Objectives

Cost-effectiveness analyses (CEAs) of interventions to prevent cervical cancer require estimates of the health-related quality of life (HRQoL) effects of screen tests and subsequent treatment. The results of such simulation analyses can be highly sensitive to the HRQoL weights employed. Accordingly, accurate assessment of HRQoL is essential for the generation of reliable CEA estimates. We reviewed the literature regarding HRQoL in cervical prevention and management in order to appraise the current evidence regarding this important input to CEA.

Methods

We searched the MEDLINE, Scopus and EconLit databases for studies that estimated HRQoL in cervical cancer prevention and management published January 1995-December 2015. The primary inclusion criterion was for studies that assess HRQoL using the EQ-5D instrument. Data were abstracted from eligible studies on setting, elicitation group, sample size, elicitation instruments, health state valuations, study design and follow-up. We assessed the quality and comparability of the studies with a particular focus on the HRQoL reported across states and groups.

Results

Fifteen papers met the inclusion criteria. Most used patient elicitation groups (n=11), 2 used the general public and 2 used a mix of both. Eight studies were cross-sectional and seven were longitudinal in design. Six studies used both the EQ-5D-3L and the EQ-VAS together with other measures of overall HRQoL or condition-specific instruments. Studies employing both the EQ-5D and specific measures of anxiety found that the EQ-5D tended to be insensitive for differences in anxiety scores detected by alternative instruments. Extensive heterogeneity was observed across study characteristics.

Conclusion

Our results reveal the challenges of sourcing reliable estimates of HRQoL for use in CEAs of cervical cancer prevention. The EQ-5D appears insufficiently sensitive for some health states. Research will be required to determine if the adoption of the more recent five-level EQ-5D-5L enhances sensitivity over the three-level EQ-5D-3L employed in the literature reviewed here. Another, more general problem is the
paucity of HRQoL estimates for many health states and their change over time. There is scope for more detailed longitudinal analysis of the HRQoL burden of cervical cancer related healthcare interventions.
CLINICAL & COST-EFFECTIVENESS OF HPV PRIMARY SCREENING & DUAL-STAIN CYTOLOGY IN THAILAND

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Background / Objectives

In Thailand, cervical cancer is a leading cause of death among women. The Ministry of Public Health is focused on reducing cervical cancer with national screening efforts, one of which has increased access to pap tests. The burden of cervical cancer remains high, however. This study seeks to evaluate the clinical and cost-effectiveness of HPV DNA primary screening as compared to pap primary algorithms, from a payor perspective, with several triage strategies, including p16/Ki-67 dual-stain cytology and colposcopy.

Methods

A Markov model was used to compare 4 strategies for women ages 30-65 (per country recommendations), across a 100-yr horizon, assuming a 5-yr primary screening interval for the strategies evaluated: 1) HPV DNA primary testing every 5 yrs with pooled high-risk positive results sent to colposcopy; 2) HPV primary testing with pooled high-risk positive results triaged with p16/Ki-67 dual-stain cytology; 3) pap primary with ≥ASCUS results to colposcopy; 4) pap primary with ≥ASCUS results triaged with p16/Ki-67 dual-stain cytology. Values for HPV DNA testing and pap screening sensitivity and specificity were obtained from the Addressing THE Need for Advanced HPV Diagnostics (ATHENA) trial. Dual-stain cytology data are from the Primary ASCUS LSIL Marker Study (PALMS) and ATHENA trials. Costs were derived from a national tertiary care hospital in Bangkok. Costs and quality adjusted life-years (QALYs) were discounted at 3.5% annually. Sensitivity analyses were conducted to assess impact of cost and clinical inputs on incremental cost-effectiveness ratios and outcomes.

Conclusion

HPV DNA primary screening with colposcopy triage offers comparable cost-effectiveness to HPV DNA primary screening with dual-stain cytology triage. For pap primary testing, colposcopy triage was dominant to dual-stain triage due to the low cost of colposcopy and pap in Thailand. All HPV strategies were cost-effective ($20,000 US threshold), but in Thailand, the convention is to use a GDP threshold closer to $6000, thus testing more than every 5 yrs, though cost-effective, may not be acceptable. Because HPV primary testing offers greater sensitivity than pap, such
strategies may provide greater value when longer screening intervals cannot be avoided. Notably, HPV DNA primary testing with dual-stain cytology triage represents the optimal strategy to reduce cervical cancer, though this would require investment in tests and triage. All HPV primary strategies modeled allow for early detection of cervical precancer and cancer, reduction in mortality, and lower treatment costs. These factors will grow even more important as Thailand works to implement a sustainable national screening program.
Cost analysis of Human Papillomavirus related cervical diseases and genital warts in Swaziland

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Background / Objectives

Human papillomavirus (HPV) has proven to be the cause of several severe clinical conditions on the cervix, vulva, vagina, anus, oropharynx and penis. Several studies have assessed the costs of cervical lesions, cervical cancer (CC), and genital warts. However, few have been done in Africa and none in Swaziland. Cost analysis is critical in providing useful information for economic evaluations to guide policymakers concerned with the allocation of resources in order to reduce the disease burden.

Methods

A prevalence-based cost of illness (COI) methodology was used to investigate the economic burden of HPV-related diseases. We used a top-down approach for the cost associated with hospital care and a bottom-up approach to estimate the cost associated with outpatient and primary care. The current study was conducted from a provider perspective since the state bears the majority of the costs of screening and treatment in Swaziland. All identifiable direct medical costs were considered for cervical lesions, cervical cancer and genital warts, which were primary diagnoses during 2015. A mix of bottom up micro-costing ingredients approach and top-down approaches was used to collect data on costs. All costs were computed at the price level of 2015 and converted to dollars ($).

Results

The total annual estimated direct medical cost associated with screening, managing and treating cervical lesions, CC and genital warts in Swaziland was $16 million. The largest cost in the analysis was estimated for treatment of high-grade cervical lesions and cervical cancer representing 80% of the total cost ($12.6 million). Costs for screening only represented 5% of the total cost ($0.9 million). Treatment of genital warts represented 6% of the total cost ($1million).

Conclusion

According to the cost estimations in this study, the economic burden of HPV-related cervical diseases and genital warts represents a major public health issue in Swaziland. Prevention of HPV infection with a national HPV immunization programme for pre-adolescent girls would prevent the majority of CC related deaths and associated costs.
References


Cancer Registry of Norway (Norway), Norwegian Cancer Society (Norway)

Background / Objectives

Norway has an organized national screening program against cervical cancer, where all women 25-69 years are recommended 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 in this age group was at its peak, at 73.0 percent. The coverage rate has decreased continuously since, to an all-time low in 2012, at 52.7 percent. By connecting different actors concerned with the low coverage, the idea was to create a yearly 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 focused on young women and included: short films featuring young, female Norwegian celebrities, editorial pieces in the magazine Det Nye, press coverage in other mainstream media, blog and social media activity with the hashtag #sjekkdeg. The campaign was followed up with a new campaign September 2016. 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

By the end of 2016 the number of registered screening tests among women in the age group 25 to 29 increased by 10,886. The 3.5 year screening coverage, increased from 55.9 percent in 2014 to of 62.1 percent at the end of 2016. There was also an increase in 34,109 women attending screening in the whole screening population between 25-69 years from 66.5 to 68.8 percent.

Conclusion

The raised awareness on cervical cancer in Norway has contributed to increased attendance to the screening program. It is reasonable to think that a large proportion of this increase can be attributed to the #sjekkdeg-campaign. 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 two years of the campaign has ensured the parties in the collaboration that the work should continue, with new campaign periods.
A NATIONAL SURVEY OF CANADIANS ON HPV: COMPARING KNOWLEDGE, BARRIERS AND PREVENTIVE PRACTICES OF PHYSICIANS TO THOSE OF CONSUMERS

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Background / Objectives

This Canadian survey of physicians and consumers aimed to explore similarities and differences in knowledge, barriers and preventive practices about HPV between the two groups.

Methods

General Practitioners (GP) (n=337) and Obstetrician Gynaecologists (OB/GYN) (n=81), vaccinated women (VW) (n=337) and unvaccinated women (UW) (n=802) aged 18-45, and men (M) (n=200) aged 18-26 were surveyed in May and June 2016 using an online questionnaire. A probability sample of the same size would yield a margin of error of +/- 4.8% for physicians and +/-2.7% for consumers, 19 times out of 20. Two posters with more detailed individual information about both groups were presented at the IPVS meeting in Cape Town, South Africa in March 2017.

Results

83% GPs recommend or administer HPV vaccine to adults. 93-98% of consumers said doctors are trustworthy sources of information. 99-100% of physicians compared to VW (93%), UW (85%) and M (59%) somewhat or strongly agree that vaccination is an important aspect of disease prevention. A higher proportion of patients were concerned about vaccine safety (VW (26%), UW (40%) and M (36%)) than were physicians (5-11%). 58-61% of consumers were generally cautious about taking any vaccine. Cost was seen as the highest barrier to getting vaccinated by 90-95% of physicians; however only 18-20% of consumers considered cost a barrier. Consumers accurately answered a majority of questions about HPV, however physicians rated consumers’ understanding of HPV to be low (11% very good and 48-56% somewhat good knowledge). Among those already vaccinated, VW (30-34%) and VM (13-31%) said physician recommendations/discussions did motivate them to be vaccinated. In the unvaccinated group, UW (38-55%) and UM (49-57%) said physician recommendations and discussions would motivate them to
be vaccinated. 60-66% of physicians say they routinely discuss HPV vaccination with patients.

**Conclusion**

Divergent views about HPV knowledge, barriers and preventive practices exist between physicians and consumers. These divergent views should be considered and addressed during physician education and consumer counselling.
Vaccinating against human papillomavirus is not associated with risky sexual behaviours among men who have sex with men in Australia

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Background / Objectives

A recent meta-analysis has concluded that vaccinating against human papillomavirus (HPV) does not lead to risky behaviours among females but there has been no studies examining this association among men who have sex with men (MSM). We aimed to examine the association between sexual behaviours and HPV vaccination status among men who have sex with men.

Methods

We analysed MSM aged 16-40 years attending the Melbourne Sexual Health Centre (MSHC), Australia, for their first visit in 2016. Chi-squared test was used to examine the differences in sexual behaviours (e.g. number of male partners and condom use in last 3 and 12 months) between vaccinated and unvaccinated MSM.

Results

A total of 1332 MSM were included in the analysis with a median age of 27 (IQR 23-31). Six percent (n=81) of MSM had been vaccinated against HPV. The median number of male partners in the last 3 and 12 months was 2 (IQR 1-5) and 5 (2-10), respectively. The proportion of men used condoms always in the last 3 and 12 months was 39.2% (n=797) and 36.5% (n=774), respectively. There were no significant differences in number of partners and always condom use in both last 3 and 12 months between vaccinated and unvaccinated MSM (p>0.05).

Conclusion

Vaccinating against HPV is not associated with increased number of sexual partners and condomless anal sex practice among MSM, particularly among sexually-active men attending a sexual health service.
SAFETY MESSAGES INCREASE MOTHERS' WILLINGNESS TO VACCINATE AGAINST HPV: A RANDOMIZED TRIAL

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Background / Objectives

U.S. HPV vaccination rates are well below national targets and there is a need to identify messaging approaches that can increase acceptance of HPV vaccine. The objective of this study was to determine if messages about the relative safety of HPV vaccination and the strength of the recommendation increased mothers' willingness to vaccinate against HPV.

Methods

1,097 mothers of 9-13-year-olds living in the U.S. completed a national Web-based survey in August 2014. The analyses presented here focused on the 63.9% (n=701) who reported no HPV vaccine administration. The study used a 3x2 randomized between-subjects design (strength of recommendation x safety information). Illustrated vignettes depicted one of 3 levels of provider recommendation strength (brief mention; strong recommendation; strong recommendation + personal disclosure of vaccination of own children), and either the presence or absence of information regarding the relative safety of vaccination compared to common daily activities (e.g., playing soccer). The outcome was willingness to have the child receive HPV vaccine, measured on a continuous sliding scale ranging from 0 (definitely would not) to 100 (definitely would). Perceived benefits of vaccination were assessed with 5 items administered prior to viewing the intervention and included as a covariate in the analysis of covariance (ANCOVA).

Results

Overall mean willingness to receive HPV vaccine was 59.7 (SD = 35.4). ANCOVA indicated that provision of relative safety information increased willingness to vaccinate (M=63.1 vs. M=56.2; F=7.0, p<.01). Perceived benefits were also significantly related to willingness to vaccinate against HPV (F=214.9, p< .001). Strength of recommendation and child sex were not associated with willingness to vaccinate and there were no significant interactions.

Conclusion

Our results suggest that provider communication about the relative safety of HPV vaccine and the benefits of vaccination in general may increase rates of HPV vaccine acceptance. While strength of recommendation did not have an effect, this may have been due to the difficulty associated with replicating a personal physician's recommendation via an online survey. As a next step it will be important to test the
relative safety messaging in settings where HPV vaccine can be administered and the effects on vaccine uptake can therefore be evaluated.
TRY THIS AT HOME: RAPID RESPONSE COALITION BUILDING AND EVIDENCE-BASED ADVOCACY.
CASE FROM INDIANA, U.S.A.

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Background / Objectives

Reminder-recall letters (RR) are an effective means of increasing vaccination in the United States. However, opposition to HPV vaccination, which is viewed by many as a sexual health intervention, can lead to efforts to thwart RR systems through changes in public health policy. When opposition emerges, however, there is a ‘window of opportunity’, when coalitions can form to provide important evidence-based communication to change the course of policy development.

Methods

Using a case study approach, we examine one example of a coalition’s emergence and its efforts to provide rapid evidence-based advocacy in the U.S. state of Indiana. Data inform a point-in-time analysis of state RR communications for HPV vaccination, HPV vaccination completion, opposition emergence, evidence-based advocacy and policy activity. The study time period is from Jan 2012-May 2017.

Results

The Indiana State Department of Health (DOH) initiated RR letters to increase HPV vaccination uptake for girls in 2012, with subsequent RR cycles for both girls and boys annually. Vaccination completion rates steadily increased, reflecting the RR intervention. Opposition emerged in 2015, following a failed state legislative bill to establish an 80% HPV vaccination completion goal, with the focus of stopping the RR program. Executive policy from the state government ensued in an effort to undermine the RR effort. Within 24 hours of the attempt to shut down the RR program, a coalition emerged with a rapid, coordinated advocacy response using social media and news outlets. This coalition, including groups from the academic and public health communities, continues today and works with the new government (new governor and legislative committee chair). The RR intervention also continues, and a new legislative policy is under consideration to allow the DOH to establish a strategic plan to reduce HPV related cancer.

Conclusion

Broad-based, coordinated and rapid communications by community partners with public health evidence to policy makers can have a positive policy impact. The ability
to identify partners and leaders, and to coordinate communications is crucial to an effort’s success. Sustained partnerships are helpful, but even in the case of Indiana, a nascent group can also be effective if coordinated, on message and timely. These efforts must be based in the community and not in government or industry to be effective.
SCHOOL NURSES’ ATTITUDES TOWARDS AND EXPERIENCES OF AN HPV VACCINATION PROGRAMME

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Background / Objectives

Healthcare providers have an important role for the HPV vaccination programme to be successful. In Sweden one in five girls is not vaccinated against HPV. A population based study among Swedish school nurses who vaccinated against HPV at the early start of the national vaccination programme in 2013 [1], showed that most nurses were in favour of the vaccination programme. However, the majority had experienced difficulties. Most nurses had been contacted directly by parents who were concerned regarding the vaccine safety and effectiveness. The aim of this study was to investigate school nurses’ attitudes towards and experiences of HPV vaccination four years after its implementation. It was hypothesised that school nurses had more favourable attitudes towards the vaccination programme, perceived less barriers with the vaccinations and had higher level of perceived knowledge about HPV vaccine compared to our previous study in 2013.

Methods

School nurses (n=736) from all counties in Sweden completed a questionnaire in spring in 2016.

Results

Overall, the school nurses had more favourable attitudes towards the HPV vaccination programme (p=0.015) and reported less barriers (p<0.001) compared to the study in 2013. More than half of the nurses (n=415, 56%) strongly agreed that boys should also be offered the vaccine (p<0.001). There were no differences in school nurses’ perceived knowledge about HPV in order to inform and to answer questions about the vaccine to the girls or to the parents. More than half of the nurses (n=409, 56%) reported that they needed more education about HPV. Almost all nurses (n=659, 90%) had been contacted by parents with questions about the vaccine, and most questions were related to vaccine safety.

Conclusion

School nurses have a more favourable attitude towards the vaccination programme against HPV compared to three years earlier, although almost all nurses had been contacted by parents with diverse questions and concerns. Thus, it is essential to
provide ongoing education and training for school nurses who are key healthcare professionals for providing information about HPV to parents and pupils.

References

The New Zealand HPV vaccination programme - the road to comprehensive access.

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Background / Objectives

This presentation will describe the development of the New Zealand (NZ) HPV vaccination programme from commencement in 2008 to 2017 with comprehensive free access as well as future plans for the NZ school programme. Challenges and coverage rates will also be discussed.

Methods

Information in this presentation is gleaned from literature review, government websites, the author's own knowledge and experience as a regional immunisation coordinator and unpublished research undertaken for her PhD.

Results

The NZ HPV vaccination programme commenced in late 2008 with a catch-up programme for girls born in 1990-1991. The school programme started in 2009 for Year 8 girls (age 12). Females could also access Gardasil in primary care until their 20th birthday. Gardasil, with a three-dose schedule at 0, 2 and 6 months was utilised for all eligible groups until January 2017.

From January 2017, enhanced access:

- Gardasil 9 replaces Gardasil (4)
- Gender neutral vaccine - offered to boys and girls in Year 8 at school
- Dose schedule change: <15s two doses at 0 and 6-12 months; 15+ three doses at 0, 2 and 6 months
- Expanded age eligibility: vaccine now funded in primary care for males and females aged 9 to 26 years

Future developments: In 2018 the Ministry of Health plans to introduce the vaccine programme to Year 7 students (age 11). This may lead to cost savings as the vaccine will be delivered concurrently with the Boostrix vaccine.

Overall coverage rates for girls have never met targets, set at 75% for three doses. Reasons for decline include safety concerns and age the vaccine is offered in school
is too young. Lower HPV knowledge is associated with poorer acceptance of the vaccine.

**Conclusion**

School based HPV vaccination started in 2009 for girls only. From 2017 NZ has a comprehensive gender neutral programme. In 2018 the vaccine will be offered in Year 7. This may lead to savings in service delivery, but may also lower acceptance, as (young) age has been a factor in parent's decision making.

Coverage rates in NZ have never been high. Reasons for decline need to be addressed in order to increase acceptance.

**References**


www.health.govt.nz

www.pharmac.govt.nz
FC 14-08
KNOWLEDGE, ATTITUDE, PRACTICE AND BEHAVIOR OF WOMEN ATTENDING GYNECOLOGICAL CLINIC TOWARDS CERVICAL CANCER AND PAP SMEAR SCREENING IN EASTERN INDIA

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Background / Objectives

In an effort to decrease the toll of cervical cancer, by its knowledge, prevention and treatment services in the community, we assessed the knowledge, attitude, and practice regarding cervical cancer screening among Eastern Indian women.

Methods

A total of 481 women were randomly selected from those who visited the outpatient clinic at MAGS Medical & Research Center, Kolkata, India irrespective of reason(s) for the visit. A pre-tested structured questionnaire covering socio-demographic characteristics, knowledge, attitude, and practice related to cervical cancer screening was administered.

Results

We found a significant lack of awareness regarding cervical cancer and its screening methods in Indian women. Only 24.5% of them had ever heard of cancer cervix and this was quite low when compared to other developed and many of the developing nations. During analysis the mean age of study group was found to be 44.29±10.036 years, 95.2% were married with mean age at marriage being 22.71±2.99 years and mean parity being 1.82. Although, depth of knowledge regarding Pap smear and cervical cancer was also found to be quite shallow in this study but it was significantly higher in those with higher educational level and higher income group. Attitude towards Pap smear test was found to be positive as more than two third of the participants aware of the test assumed it to be beneficial. The role of education and economic stability was also established in regard to perceived benefits of pap smear and this distribution was significant statistically (P value 0.049 and 0.015). It was seen in this study that the positive attitude towards the test was translated into right practice and behavior.

Conclusion

This study revealed the limited knowledge of Indian women about the susceptibility of cervical cancer, and the necessity of cervical cancer screening among the women. Inadequate public health education, lack of patient-friendly health services, socio-cultural health beliefs, and personal difficulties were the most salient barriers to screening.
DETERMINANTS OF HPV E6-E7 MRNA OVEREXPRESSION IN WOMEN HPV DNA POSITIVE - PRELIMINARY RESULTS FROM NTCC2 STUDY

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Background / Objectives

Cervical cancer screening by Human Papillomavirus (HPV) DNA testing with cytology triage is more effective than cytology based screening. Compared to cytology, the HPV DNA test shows higher sensitivity, which allows better protection and longer screening intervals, although a lower specificity which makes it necessary to triage the women resulted positive. We have been conducting a large randomized clinical trial (New Technologies for Cervical Cancer 2 [NTCC2], NCT01837693)(1) within organized population-based screening programs in 5 Italian regions using HPV DNA as the primary test, with the aim of evaluating the HPV E6-E7 mRNA test (Aptima HPV assay, Hologic) and the p16/ki67 double staining (CINtec plus test, Roche) as the triage test in comparison to cytology.

Methods

Women were tested with HPV DNA assay (Cobas 4800 HPV assay, Roche, or Hybrid Capture 2 (HC2), Qiagen). Those positive were triaged with cytology and tested for mRNA and p16/ki67 double staining. Women with positive or inadequate cytology were referred to colposcopy, while those with negative cytology were randomised to immediate colposcopy or to 1-year HPV re-testing. Women will be followed up for at least 5 years, until the next screening round. Here the baseline results of mRNA positivity according to age, cytology, HPV type, and HPV viral load, are presented.

Results
More than 42,000 25-64yo women have been recruited from April 2014 to April 2017. Here are included data from 35,877 samples collected up to June 2016; 2,651 (7.4%) were HPV DNA positive. Up to now, 2,453 samples have been tested also by Aptima, and 1,649 (67.2%, 95% CI 65.4-69.2) gave a positive result. Positivity was similar in all age groups, and it was higher in women with positive cytology (82.7%, 95%CI 79.0-86.0; 93.5%, 95%CI 88.3-96.8, for low and high grade, respectively) than in those with negative cytology (60.8%, 95%CI 58.5-63.1) and in women HPV16 infected (81.8%, 95%CI 76.4-86.4) compared to those infected by other high risk types (72.6%, 95%CI 69.8-75.3). Finally, positivity increased with HPVDNA viral load from 10.6% (95%CI 6.9–15.3) for women with HC2 relative light unit ratio (RLU) between 1 and 1.99, to 85.0% (95%CI 82.2–87.6) for women with HC2 RLU >10.

Conclusion

If used as a triage test, the mRNA test, due to the observed positivity rate, would determine a 5% immediate colposcopy referral, compared to 2% with cytology triage. Thus, the number of women referred to colposcopy would increase if the 1-year referral for HPV DNA-positive/mRNA-negative cases is maintained. Only longer interval for these women might make triage by this test more efficient than by cytology, as long as the mRNA test shows very high sensitivity.

References

A THREE-TIERED SCORE FORMAT FOR KI-67 AND P16INK4A IMPROVES CONSISTENCY AND VALIDITY OF GRADING CIN LESIONS

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Background / Objectives

Accurate histological grading of cervical intraepithelial neoplasia (CIN) is essential for clinical management. However, CIN grading has a moderate inter- and intra-observer agreement. We investigated the reproducibility of the performance of a score system based solely on the cumulative score value of the biomarkers Ki-67 and p16ink4a (immune-score), and compared the results to consensus pathologist CIN grading based on slides stained for H&E, Ki-67 and p16ink4a.

Methods

Three expert gynaeco-pathologists received H&E slides of 115 randomly selected cervical tissue specimens, selected the most abnormal area and rendered a diagnosis (diagnosis 1). At a later time point, the individual pathologists independently scored corresponding Ki-67 and p16ink4a immunostainings by a three-tiered immune-score system. Next, a diagnosis was made based on both the H&E and immunostainings (diagnosis 2). The consensus diagnosis 2 was used as the Gold Standard. Consistency of diagnosis 1, 2 and immune-score was determined by Spearman Correlation coefficients, Kappa values and absolute agreement between pathologists. Validity of the diagnosis 1, 2 and immune-score was determined by sensitivity and specificity for CIN2+ and CIN3+ graded by the Gold Standard diagnosis.

Results

Gold Standard diagnoses revealed 35 specimens without dysplasia, 20 CIN1, 17 CIN2, 22 CIN3 and 21 specimens with SCC. The highest consistency between pathologists was found for the immune-score, with a Spearman correlation coefficient of 0.907, and a maximum Kappa of 0.829 and absolute agreement of 92%. Immune-score showed a higher sensitivity for Gold Standard CIN2+ and CIN3+ than diagnosis 1 and 2, with a sensitivity for marker scores 3 to 6 being higher than 97.0% and an increase in specificity from 70.0% to 85.9%, respectively.

Conclusion
CIN grading based on a simple three-tiered Ki-67 and p16^{ink4a} immune-score has a higher reproducibility and accuracy in terms of consistency and validity than classical histological and immunohistochemical CIN grading. Moreover, this immune-score defines more accurately where on the trajectory of development of cervical cancer via CIN1 to CIN3 the cervical lesion is situated. This is important for the clinician to decide on cervical treatment or a wait and see policy.
P16/KI67-BASED TRIAGE FOR HISTOLOGIC HSIL-RISK WOMEN IN 12-18 FOLLOW-UP: P16/KI67 TWICE-POSITIVITY AND COLPOSCOPY FIRST-NEGATIVITY.

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Background / Objectives

All world-wide recommended algorithms in cervical cancer secondary prevention have limitations in precancers detection. Simultaneous co-expression of anti-proliferative and proliferative proteins in p16/Ki67 test is clinically used to select high-grade cervical intraepithelial lesions. We evaluated whether twice-positivity p16/Ki67 test – in first test and in one year follow-up – can improve detection of these.

Methods

8824 automated proceeded LBC (a study period 08/2015 – 04/2017), including 2063 as cotesting with DNA high-risk HPV, have been performed in secondary cervical cancer prevention. Immunocytochemical p16/Ki67 double staining was done in 372 cases using automated preparation system. 180 women with ASC-H or higher or ASC-US/LSIL cytology and with HPV-positive status were referred to colposcopy with biopsy. 35 patients with histological LSIL or less (biopsy first-negativity), reached follow-up cotesting with p16/Ki67 test and biopsy in 12-18 months.

Results

Diagnostic value of twice-positivity p16/Ki67 test for histologic HSIL (hHSIL) in the second follow-up biopsy was evaluated. Follow-up p16/Ki67 test was positive in 11 women – 8 hHSIL and 3 histologic LSIL cases were diagnosed in biopsy. 1 hHSIL was p16-Ki67 twice-negative. Sensitivity/specificity/PPV/PPV of p16/Ki67 for hHSIL in the second biopsy was 89/88/73/96 (CI 95%) respectively.

Conclusion

A twice-positive p16/Ki67 test can be a precise biomarker in triage patients for hHSIL-risk in cervical cancer screening. In women with abnormal screening test results after first biopsy, p16/Ki67 could be sufficient as alone diagnostic test in referring to follow-up biopsy in 12-18 months.
A NOVEL WHOLE GENOME SEQUENCING METHOD TO ACHIEVE A COMPREHENSIVE MAP OF ALL HPV16 INTEGRATION SITES ACROSS THE HUMAN GENOME

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Background / Objectives

Our study will have 3 main goals. 1) Through our novel approach utilizing the latest in NGS techniques, we will extensively map all HPV integration events across the human genome for each cancer and pre-cancer patients in our initial cohorts. 2) Using in-house developed analytic software, we will combine each patient’s integration maps and identify integration patterns that could be used for future targeted approaches, and 3) the whole genome human data will also be used to identify all chromosomal rearrangements within the tumors and the proximity of these events to the cataloged integration sites.

Methods

For this study we have chosen to use 10X Genomics Technology in combination with Illumina whole genome sequencing to provide us with the most accurate, phased human genomes. Our pilot study consists of 16 HPV16+ women (8 cancers, 4 pre-cancers (CIN3) and 4 control HPV16+ non-cancer samples (CIN2)).

Results

We have performed whole genome sequencing on all 16 women from our pilot study. All 16 of the pilot samples have been processed through our cloud based analytic pipeline that aligned each sample to an HG19-HPV16 hybrid reference, for each sample their HPV16 integration sites have been recorded as well as all other chromosomal abnormalities have been identified and recorded.

Conclusion

We have developed a method that combines the latest in NGS technology to create a comprehensive list of HPV integration and chromosomal abnormalities for each of the 16 women in our first pilot study. This data will be used for potential development of targeted assays.
WHOLE EXOME SEQUENCING TO FIND NEW BIOMARKERS FOR DETECTION OF CIN3

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Background / Objectives

Our team has developed a methylation classifier to detect CIN3 that could be used routinely as a molecular diagnostic tool. Although this classifier already has good sensitivity and specificity our aim was to improve it further. We used whole exome sequencing to find new biomarkers to add to the classifier.

Methods

The study was designed to compare the baseline and dyskaryotic samples of HPV positive women who developed CIN3 (cases) to women who did not (normal controls) and to HPV negative women (negative controls). The samples at baseline did not show any sign of dyskaryosis. From the archived material of the ARTISTIC trial, we selected 27 cases (2 samples per case, one at baseline, one at time of diagnosis) that were matched to 27 normal controls by age and HPV type to the cases. Twelve negative controls were also randomly selected. In total, we sequenced the whole exome of 93 samples on an IonProton using the IonAmpliSeq Exome RDY kit. We developed an in-house bioinformatics pipeline.

Results

First, we validated our bioinformatics pipeline on the top 29 candidate single nucleotide polymorphisms (SNPs) using Sanger sequencing. All SNPs were successfully validated which showed that the sequencing and analytical methods were appropriate. Then, using a principal component analysis with both germline and somatic mutations, we showed a clear separation between both types of controls and the cases. We found that the cases harboured mutations at baseline and time of CIN3 diagnosis that were not present in any of the controls. We are now in the process of validating the top 200 variants using Fluidgm and Illumina technologies. More details of our ongoing investigations will be reported.

Conclusion

We have validated the use of whole exome sequencing on the IonProton platform to identify new biomarkers for detection of CIN3 and produced a list of 200 candidate SNPs to improve our classifier.
MICRORNA DETECTION IN CERVICAL SCRAPES ALLOWS FOR THE TRIAGE OF HPV-POSITIVE WOMEN IN CERVICAL SCREENING

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Background / Objectives

Screening for cervical cancer by primary high-risk HPV (hrHPV) testing has just been introduced in the Netherlands. Because hrHPV testing also results in the identification of women with clinically irrelevant transient hrHPV infections, additional triage markers are required to identify women at risk of high-grade cervical intraepithelial neoplasia (CIN) or cancer. We expect microRNAs (miRNAs) to provide promising triage markers, since they are 1) often deregulated in cancer and 2) easily detectable in small amounts of clinical material. We previously identified 8 miRNAs with altered expression in cervical (pre)cancer due to either methylation-mediated silencing or chromosomal alterations. In this study, we evaluated the clinical value of the 8 miRNAs to triage hrHPV-positive women in cervical screening.

Methods

Quantitative TaqMan RT-PCR was used to determine expression levels of the 8 candidate miRNAs in RNA isolated from cervical scrapes of hrHPV-positive women without disease (n=66), with high-grade CIN (n=121), cervical squamous cell carcinoma (SCC, n=29) and cervical adenocarcinoma (AC, n=9).

Results

Six out of 8 miRNAs showed significantly (p < 0.05) differential expression between scrapes of hrHPV-positive women with and without high-grade CIN and cancer. Using logistic regression analysis a miRNA classifier was built, which detected a major subset of high-grade CIN (~65%), all but two SCC (93%) and all AC (100%).

Conclusion

Altered miRNA expression is detectable in hrHPV-positive cervical scrapes of women with underlying high-grade CIN and cancer. Our miRNA classifier provides promising results for the triage of hrHPV-positive women.
Co-expression of HPV E6, E7 mRNA and PD-L1 in Cervical Cytology Samples: Prognostic Implications

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Background / Objectives

The immune system has been shown to control HPV infection in some women and not in others leading to pre-cancerous lesions and subsequently cancer. The relatively high regression rate of cervical intraepithelial lesions (CIN) has similarly been attributed to engagement of the immune response directed against neoplastic cells. Recent advances in immune-oncology have shown the dramatic effects of PD-1/PD-L1 inhibitors in epithelial tumors including squamous cell carcinoma and adenocarcinoma, the major cancer subtypes in the female genital tract. Here, we present a novel assay that combines RNA in situ hybridization for HPV E6, E7 mRNA and PD-L1 cell surface staining on squamous cells in liquid-base cervical cytology specimens. This assay could provide actionable data supporting therapeutic options.

Methods

ThinPrep liquid based cervical cytology samples were obtained from both normal patients and patients with HSIL cervical cytology. Cells were isolated and hybridized in suspension for HPV E6, E7 mRNA using the HPV OncoTect 3Dx (CE-IVD) kit. Following post-hybridization washes, cells were stained with PD-L1 using the OncoTect iO (CE-IVD) kit then counterstained with cell cycle reagent. Cells were analyzed on a CytoFlex flow cytometer (Beckman Coulter).

Results

PD-L1 expression was low in the normal HPV negative samples and variable in the HSIL samples using a standard 1% cut-off for positivity in squamous cells. Of interest, PD-L1 expression was higher in cells expressing HPV E6, E7 mRNA compared to cells lacking transcriptional activity.

Conclusion

PD-L1 expression is variable in samples with abnormal cervical cytology. The prognostic implications of PD-L1 expression in cervical pre-cancer remains to be determined, however, we present in this study a means for fine quantification of PD-L1 expression in both HPV transcriptionally active and normal cells. This combination disease markers should facilitate further studies of the role in progression or regression of cervical pre-cancer.
Association between PD-L1 mRNA expression and HPV infection in cervical adenocarcinoma and squamous cell carcinoma

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Background / Objectives

PD-L1 expression in different subtypes of cervical cancer is largely lacking. We investigated the expression of PD-L1 mRNA in human papillomavirus (HPV)-negative and HPV-positive adenocarcinoma (CADC) and squamous cell carcinoma (SCC) to determine its prevalence and the association between PD-L1 expression level and HPV infection.

Methods

RNA ISH was performed with an RNAscope 2.5 (Advanced Cell Diagnostics) according to the manufacturer’s instructions. 85 cases of formalin-fixed paraffin embedded tissue sections were evaluated for mRNA expression of PD-L1 and HPV. All cases were tested HPV DNA using WTS-PCR and multi-infected cases were tested HPV DNA using LCM-PCR. HPV and PD-L1 expression were visualized with standard bright-field microscopy and reviewed by two pathologists. Positivity was defined as 1 to 3 spots in one target cell (20-40X), scored 1. If the signal was 4 to 10 spots in one cell, more than 10 spots in one cell and lower than 10% cells are with clustered spots or more than 10 spots in one cell and more than 10% cells are with clustered spots will be scored 2, 3 or 4, respectively. To obtain significance in the difference between groups was performed by Chi-square test using SPSS 24.0 software.

Results

Our results showed that the PD-L1 positivity was found in 20 out of 71 (28.2%) CADC cases, while it was found in 4 out of 14 (28.6%) SCC cases. PD-L1 mRNA was expressed in 11 out of 32 (34.4%) HPV mRNA positive CADC and SCC cases and that was 13 out of 53 (24.5%) HPV mRNA negative cases. In HPV DNA positive group, PD-L1 positivity was found in 15 out of 50 (30.0%) cases. In HPV DNA-negative group, PD-L1 positivity was found in 9 out of 35 (25.7%) cases. Above all, PD-L1 positivity was slightly higher in SCC and HPV-positive group than that of CADC and HPV-negative group. But no significant differences were observed in these groups.

<table>
<thead>
<tr>
<th>HPV/PD-L1</th>
<th>RNA Scope PD-L1 score (case/%)</th>
<th>Total</th>
</tr>
</thead>
</table>

Table 1 PD-L1 mRNA score for RNAscope ISH
### Conclusion

We didn’t observe differences of PD-L1 mRNA expression between SCC and CADC or HPV+ and HPV- cervical cancer. This may due to limited SCC cases. Further research can be done to investigate the association between HPV and PD-L1 mRNA expression.

### References

KERATIN 17 (K17) IS A PROGNOSTIC BIOMARKER OF CERVICAL CANCER: ENDOCERVICAL GLANDULAR NEOPLASIA.

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Background / Objectives

Although p16INK4a and other diagnostic biomarkers have been established as sensitive and accurate diagnostic biomarkers for cervical squamous cell carcinoma (SCC) and endocervical adenocarcinoma, they have limited power to predict the survival of patients following the diagnosis of cervical cancer. By contrast, we discovered by laser capture microdissection and mass spectrometry, that keratin 17 (K17) could predict the overall survival of patients with squamous cell carcinoma (SCC) more accurately than grade, stage, or any other clinicopathologic features1,2. K17 status, however, has not been previously evaluated in glandular neoplasms of the endocervical mucosa. Based on the concept that both squamous and glandular lesions of the cervix are driven by similar pathogenetic mechanisms, we hypothesized that K17 overexpression could also be a prognostic biomarker for endocervical glandular neoplasia.

Methods

Cases of endocervical adenocarcinoma, adenocarcinoma in situ (AIS), benign glandular lesions, and normal endocervical mucosa were selected from the archival collections of formalin-fixed, paraffin-embedded tissue blocks from the Departments of Pathology at Stony Brook Medicine and the University of Massachusetts. Immunohistochemical staining for K17 was performed by an indirect immunoperoxidase method and K17 expression was scored based on the PathSQ score, the proportion of cells that showed strong (2+) staining.

Results

K17 was highly expressed in 21/32 (65.6%) cases of adenocarcinoma in situ (AIS) and in 75/90 (83%) of adenocarcinoma cases. K17 staining was detected in a mean of 33.94% of malignant cells and was strongest at the periphery of pseudoglandular groups and at the invasive front of tumors. K17 was not detected in benign glandular lesions but was found in subcolumnar reserve cells, most notably in microglandular hyperplasia. High levels of K17 expression were significantly associated with decreased patient survival. While the 40-89% K17+ category did not significantly differ from the <40% category (HR = 2.03, p=0.13), those with a 90%-100% K17+ scores had 3.47 times higher risk of death as compared to the risk of death for those with a <40% K17 score (p=0.01).

Conclusion
In summary, K17 expression in normal endocervix was limited to subcolumnar reserve cells while expression in the columnar endocervical epithelium was highly specific for glandular neoplasia of the cervix. Most importantly, we further determined that high K17 expression is a powerful, negative prognostic biomarker that can be used to identify patients that have the shortest survival probability following the diagnosis of endocervical adenocarcinoma.

References


THE ROLE OF FUNCTIONAL POLYMORPHISMS AS POSSIBLE MODULATORS OF REACTIVE OXYGEN SPECIES IN CERVICAL CANCER

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Background / Objectives

The aim of this study was to investigate the genetic susceptibility of functional polymorphisms involved in the regulation of reactive oxygen species (ROS) - NADPH oxidase (NOX) subunit (p22phox) and myeloperoxidase (MPO) in cervical carcinogenesis.

Methods

A total of 862 women, 312 cases (46 LGSL+ 32 HGSIL+ 234 ICC) (mean age of 46.20±13.2 years old) and 550 controls (mean age of 42.44±13.38 years old). In this population were determined the polymorphisms of p22phox (CYBA C242T, rs4673) and MPO (G463A, rs2333227) by PCR-RFLP. Statistical analyses were qui-square and binary logistic regression. The results were significant for P<0.05.

Results

In our population, we confirmed that age and smoking habits were risks factors for development of cervical cancer (OR=1.04, 95%CI [1.02-1.05], P<0.0001; OR=2.47, 95% [1.36-4.47], P=0.003, respectively). The A carriers of MPO polymorphism were about 5-fold of increased risk for cervical cancer (OR=5.41, 95% [2.15-13.64], P<0.0001), being dependent of age (OR=3.38, 95% [0.85-13.48], P=0.085) and independent of smoking habits (OR=3.85, 95% [1.33-11.11], P=0.013). In p22phox polymorphism, the TT genotype was a tendency for increased risk in cervical cancer (OR=3.57, 95% [0.85-13.48], P=0.057), being age and smoking habits dependents.

Conclusion

Epithelial lesions, target for HPV, are exposed to ROS, therefore, functional polymorphisms involved in the modulation of this species may influence the susceptibility for cervical cancer. The A carriers of MPO and CC genotype of ph22phox polymorphisms, associated with lower activity of macrophages, may contribute to higher susceptibility to cervical carcinogenesis.
INTER- AND INTRA LABORATORY QUALITY MONITORING OF HPV TEST PERFORMANCE IN THE DUTCH CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

From January 2017 primary screening for cervical cancer in the Netherlands has switched from cytology to HPV based screening. The programme expects to examine well over 500,000 samples per year distributed over five CCKL or ISO15189 accredited laboratories. To achieve uniform performance between the five sites all laboratories are equipped with identical Roche Cobas4800 systems, three per laboratory. Furthermore, the Cobas 4800 HPV test kit production lot, laboratory reagents and assay procedures are all standardized between the laboratories.

Methods

To develop a quality control programme to monitor and continuously ensure the quality of HPV based screening results within and between the Dutch screening laboratories and to compare their performance with international peer laboratories outside the Dutch screening network. This integrated quality monitoring system is based on:

A verification and release programme for acceptance testing of equipment upon installation and upon repair or major service. In addition, this programme is used to test and release (new lots of) critical reagents.

2. A run control programme with a manufacturer-independent control sample in each HPV run.

3. External Quality Assessment to monitor inter-laboratory performance by participating in both international inter-laboratory comparisons with proficiency panels and in national comparisons based on inter-laboratory exchange of clinical materials from the screening program.

Results

Dedicated HPV control panels were designed and used for acceptance of systems and kits prior to the start of the programme. These panels have been instrumental to achieve the required level of performance standardization within and between
laboratories. Furthermore, the results to be presented demonstrated that the observed inter-run, inter-system as well as inter-laboratory variations in the Ct values of the results were small and well within acceptable ranges.

From the start of the screening programme an independent run control sample was included in each assay run. This control is completely independent from the assay supplier both in design, development, production and result interpretation. The independent run control, called User Defined External Control (UDEC), is examined in every run in each laboratory. UDEC results are used to monitor the (variation in) assay performance within and between the screening laboratories; it is not aimed for acceptance of individual runs

Conclusion

The quality monitoring programs have been implemented to monitor assay performance in the Dutch hrHPV screening network of five laboratories participating in the recently renewed national screening program for cervical cancer.
P16/KI67 DOUBLE STAINING FOR TRIAGE POSITIVE RESULTS IN PRIMARY CERVICAL CANCER SCREENING BASED ON DNA HPV TESTING.

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Background / Objectives

Human papillomavirus (HPV) DNA testing is globally recommended in primary cervical cancer screening. Effective triaging of HPV-positive women plays a crucial role for detection cancer precursors. p16/Ki67 has been studied for distinguishing of high-grade cervical intraepithelial lesion (HSIL) risk by co-expression of anti-proliferative/proliferative markers. We investigated a diagnostic value of p16/Ki67 as the second-step in HPV-based screening.

Methods

From 8824 cervical cancer screening tests (including 1718 cotesting, 345 LBC with reflex HPV and 372 p16/Ki67 tests), a group of 189 cases was selected based on 4 end-points: positive high-risk HPV status, LBC and double immunocytochemical p16/Ki67 test (DS) in automated preparation systems and performed colposcopy with biopsy as follow-up.

Results

Total number of histologic HSIL/DS positivity was 33/30 - for positive 16 or 18 types HPV (16/18HPV+) 21/20 and for positive non-16 or non-18 types (n16/n18HPV+) 12/10, for histologic LSIL was 70/23 - for 16/18HPV+ 28/12 and for n16/n18HPV+ 42/11, and for negative was 84/16 – for 16/18HPV+ 37/10 and for n16/n18HPV+ 47/6. Sensitivity/specificity/PPV/NPV of DS for hHSIL were 91/74/42/97 respectively. In retrospective analysis, total number of biopsies needed in p16/Ki67-based triage was 71 comparing to 180 in LBC-based triage.

Conclusion

p16/Ki67 can be a clinically important diagnostic test for detecting hHSIL in HPV-positive women that may reduce number of performed invasive procedures and increase patients comfort. In consequence, incorporating of p16/Ki67 test clinical algorithms could reduce total costs of secondary cervical cancer prevention.
FC 17-03
HPV-positive women with normal cytology remain at increased risk of CIN3 after a negative repeat HPV test

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Background / Objectives

In human papillomavirus (HPV) screening, a repeat HPV test is often recommended after positive HPV and normal cytology (HPV-pos/cyt-neg) but its absolute risk of cervical precancer (CIN3+) over two screening rounds needs to be assessed.

Methods

We assessed the five-year risk of HPV infection and CIN3+ in HPV-pos/cyt-neg women with a negative repeat HPV test and in double negatives (negative HPV and cytology) in the POBASCAM cohort. We obtained histology data from the Dutch pathology registry (PALGA).

Results

HPV infection risk was 20.4% (19/93) in HPV-pos/cyt-neg, repeat HPV-negative women and 3.2% (294/9,185;p<0.001) in double negatives. Corresponding CIN3+ risks were 2.0% (4/199) and 0.2% (41/18,549,p<0.001). Infection risks were also increased in type-specific analyses of HPV16,31,33,39,52,56 and 58.

Conclusion

HPV-pos/cyt-neg women continue to have an increased CIN3+ risk, also when the repeat HPV test is negative. Therefore, intervals in primary HPV screening should be determined separately for HPV-positive and -negative women.
Non-inferiority of Onclarity HPV genotyping compared with HC2 in a German HPV-screening pilot project (WOLPHSCREEN)

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Background / Objectives

Only new HPV tests that show similar sensitivity and specificity for the detection of CIN3+ as HC2 or GP5+/GP6+ should be used in cervical cancer screening programs. We evaluated the performance of Onclarity for the detection of HR-HPV compared with HC2 in a German HPV screening program and the utility of Onclarity genotyping for the triage of HR-HPV positive women compared with p16/Ki-67.

Methods

Women attending for their 1st, 2nd or 3rd HPV&Pap screening round in WOLPHSCREEN were included. Participants were 30-70 years old, hysterectomy was an exclusion criterion. All underwent Pap smear and HC2 co-testing with 5 years intervals for women with normal findings. In 2015/16 ThinPrep samples of 4,699 participants were tested with Onclarity in a non-interventional followed by an interventional trial with 2,781 women in 2016/17. In this second phase women were called for colposcopy when Onclarity tested positive for HPV 16, 18, 31, 33, 45 or 58.

Results

The overall HR-HPV prevalence was 7.0% with Onclarity and 8.47% with HC2. Only 40/4,301 HC2 negative samples were tested positive with Onclarity (0.93%). Sensitivity of Onclarity for CIN2+ was 93% (40/43), specificity 94.2%, NPV 99.9% and PPV 10.7%. Genotyping for HPV 16, 18, 31, 33, 45 or 58 and p16/ki-67 triage showed an identical sensitivity of 79.1% for CIN2+.

Conclusion

The overall performance of Onclarity HR-HPV testing was non-inferior to HC2 in WOLPHSCREEN. Onclarity showed a better specificity than HC2 while sensitivity for CIN2+ was slightly lower. Primary screening with Onclarity and the use of genotyping to triage HR-HPV positive cases seem feasible.
Significant reduction of cervical cancer incidence within a primary HPV screening pilot project in Wolfsburg, Germany (WOLPHSCREEN)

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Background / Objectives

A number of randomized controlled trials (RCTs) showed that screening with HPV testing, either as co-testing with cytology or as stand-alone test, led to a significant reduction in cervical cancer incidence compared with cytology based screening. However, evidence for a similar efficacy outside of RCTs is still lacking. Here we report on the 11 years follow-up of the Wolfsburg primary HPV screening pilot project (WOLPHSCREEN).

Methods

26,624 women were recruited between Feb 2006 and Dec 2016. Participants had to be at least 30 years old, hysterectomy was an exclusion criterion. All underwent co-testing with Pap smear and HR-HPV testing (HC2). Women with normal findings had their next screening round after 5 years, Pap+/HC2+ case were immediately transferred to colposcopy, while cases with discordant findings had repeat testing after 12 months with referral to colposcopy in case of persistent positive findings.

Results

Overall 305 CIN3+ cases were diagnosed, including 31 invasive cervical cancers. The 5 years incidence of CIN3+ was 0.96% (95% CI: 0.85%-1.09%) in the first round and dropped significantly to 0.16% (95% CI: 0.10% - 0.26%) in subsequent screening rounds. The observed decline in cervical cancer incidence was even steeper from 0.1089% (95% CI: 0.07%-0.16%) in the first to 0.0167% (95% CI: 0.00%-0.06%) in subsequent rounds. The vast majority of CIN3+ cases were diagnosed at first colposcopy (277/305). In the second screening round the remaining cancers were explained by failure of screening tests or colposcopy, while the majority of CIN3 were classified as new lesions.

Conclusion

The observed decline of CIN3+ and invasive cervical cancers in WOLPHSCREEN gives proof that significantly better cervical cancer prevention can be achieved with HPV-testing even outside of RCTs.
EFFECTIVENESS OF SCREENING IN HPV VACCINATED WOMEN


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Background / Objectives

With the first birth-cohorts of vaccinated women reaching the age of screening its characteristics (most notable positive predictive value) will change abruptly. There is a great need to redesign an appropriate screening protocol as right tools for the future cervical cancer screening programs. We report here the baseline characteristics and safety interim results of a randomized trial assessing the impact of infrequent vs. frequent cervical screening in human papillomavirus (HPV) type 16/18 vaccinated women.

Methods

Total female 1992-94 birth-cohorts (30129) were invited to and respectively vaccinated (9482 + 2036) as early adolescents (age 12-15 years) or as young adults (age 18 years) against HPV16/18 in a community-randomized trial (EUDraCT 2007-001731-55). Women in this trial will be screened at the age of 22, 25 and 30 years for cytological screening. In one arm, representing frequent screening, the participants receive information on all the cytological findings on each visit, in the other arm, representing infrequent screening, the participants receive this information only at the of 30, information on high-grade squamous intraepithelial lesion (HSIL)s is, however, given due to ethical reasons. In 2010-13, 4660 (49.1%) and 2036 (100%) of the HPV vaccinated women attended a baseline cervical screening visit, and in 2014-2017 4018 (42.4%) and 1326 (65.1%) of these women attended the 1st round of cervical screening at the age of 22 years.

Results

Overall prevalence of HSIL and any abnormal cytological findings at the 1st screening round were: 0.3% (n=11) and 5.7% (n=220) in those vaccinated as early adolescents, and 0.2% (n=3), and 4.6% (n=58) in those vaccinated as young adults, respectively. The incidence rate from the 1st screening round of HPV16/18 and other HPV DNA are being currently analysed and will be combined with the above cytological results. Our previous results from the adolescent group at the age 18
showed HPV16/18 and HPV31/33/35/45 prevalence range from 9.9% to 10.7% and 9.2% to 10.2%, respectively.

Conclusion

Our birth cohorts attending screening, as described above, are in close to ten years ahead of comparable observational studies or monitoring of vaccination program participating birth cohorts in other affluent countries. Our study will show the first results of safety, accuracy and effectiveness of infrequent vs. frequent screening for cervical cancer safety which can pave the way to the future synergistic vaccination/screening programs.
FIRST RESULTS OF THE EU-TOPIA PROJECT: TOWARDS IMPROVED CERVICAL CANCER PREVENTION IN EUROPE

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Background / Objectives

Screening programmes vary substantially between countries and in most long-term effectiveness of screening has not yet been assessed. In 2015 the EU-TOPIA project has started. The objective of EU-TOPIA is to systematically evaluate and quantify the harms and benefits of the screening programmes for cervical, breast, and colorectal cancer in all European countries, and identify ways to improve health outcomes and equity for citizens. We will present an overview of the first result of this important EU funded project, focusing on the results for cervical cancer prevention.

Methods

First, we have harmonized key quality indicators across cancer sites and prioritized key indicators for the harms and benefits of screening. Second, we systematically evaluated literature on the effects (in terms of mortality or incidence reduction) of cancer screening across the European countries. This information will be used to validate decision models which will be used to find the optimal screening strategy. Third, we started constructing state-of-the-art decision models of the natural history of the cancers (based on the well-known microsimulation model MISCAN), using country-specific data. Fourth, a CATWOE (‘Customers, Actors, Transformation, Weltanschauung, Owner, Environment’) analysis tool was developed to identify barriers hindering implementation of optimal screening programs. This tool was filled in for six EU countries representing four European region (North, East, South and West). Fifth, the first workshop on monitoring screening programmes in a series of four workshops is planned. The aim of the workshops is to build capacity to conduct cancer screening evaluation independently.

Conclusion
We prioritized a set of 17 key quality indicators (7 benefits and 10 harms), harmonized across cancer sites. The literature search provided evidence that cervical cancer screening is proven to reduce mortality across Northern and Western Europe. However, no studies have been performed evaluating the direct effect of screening on mortality in other European regions. The CATWOE analysis identified the most important barriers and different levels of the screening programme in six exemplary countries. It was found that many barriers occur in each country/region, but also many specific to each country/region. Finally, the first workshop will be held in September 2017. Currently, already 60 persons (researchers and policymakers) registered from 25 different countries. In conclusion, important progress have been made within the EU-TOPIA project.
FC 17-08
HPV INFECTION AMONG ELDERLY WOMEN – RESULTS FROM A POPULATION BASED COHORT STUDY

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Background / Objectives

During 2012-2014 in Örebro County, Sweden, the organized screening program included women between 23 and 60. Women with normal cytology then used to exit the program at 55-60 years of age. The aim of this study was to see if HPV test (professionally collected and/or self-collected tests) could predict cervical histological changes and be used as screening option in this age group.

Methods

All women (between 55-60) with normal cytology in their exit sample in the screening program during the years 2012-2014, a total of 2030, were invited to participate in the study. All samples were genotyped for HPV DNA with CLART HPV2 (Genomica). A total of 249 of the 2030 (12.3%) women were positive for carrying any of the 35 HPV genotypes. Of these, 141/249 carried an intermediate or high risk HPV according to the IARC classification, group 1 and 2A and B. These 141 women were invited for a new visit with professional sampling, and also to use a self-sampling device, (Rovers Evalyn brush) and to have a cervical cone performed. Out of these 141 women, 99 have completed all the above these testing and also came for a follow up test 6 months after the histology sample.

Results

Concordance between self-sampling and professionally collected samples was seen in 82.4% and both test methods correlated in the same way to the histological results. Abnormal histology was seen in 19/99(19%) of the performed cones. Follow up tests after 6 months showed that 17/99(17%) of the women still had a HPV positive test.

Conclusion
Further results and conclusion will be presented at the conference, with focus on different HPV genotypes, the concordance with histological findings and HPV persistence.
IF PERSISTENT HPV INFECTION CAUSES DISEASE, WHY ARE WE NOT MEASURING IT?


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Background / Objectives

There is universal agreement that HPV is required for the development of cervical disease and that HPV-induced disease is associated with a persistent HPV infection. The introduction of organized cervical cytology screening programs has resulted in a marked reduction in cervical cancer morbidity and mortality. Here, the relative insensitivity of cytology to detect disease was offset by the serial nature of the screening paradigm, where disease [resulting from persistent infection(s)] was often detected over several screening rounds. Many countries are now shifting to primary HPV screening using extended screening intervals. As we embark on this transition, the irony is that we seem to be ignoring some basic first principles of cervical cancer screening, namely the ability to monitor persistent HPV infection directly using extended genotyping (beyond HPV 16/18).

Methods

Here we argue that extended genotyping information provides a unique and necessary insight into a woman’s risk for developing disease:

1. It enables the physician to detect persistent infections which have a higher disease risk
2. It can guide management decisions, especially in short-term follow up scenarios
3. It enables comprehensive test of cure to be performed on treated women (not just for HPV 16/18)
4. It can identify non-HPV16/18 types with an increased CIN3 risk (e.g. HPV 31, 33 and 45) versus just recording “other-high-risk” infections, enabling them to be tracked over time
5. It provides critical information for downstream “risk-based colposcopy” follow up
6. It provides important information on vaccinated women with reduced HPV16/18 disease burden

We illustrate these points using data from the BD Onclarity™ PMA and CE Mark trials and examples from recently published literature. We also demonstrate that extended genotyping overcomes one of the primary deficiencies of the 12-other HPV pool screening approach, namely the inability to accurately assess a patient’s CIN3 risk due to masking (when 2 or more HPV types with different absolute risks result in a pooled average risk that is an underestimate of the highest individual genotype risk).
Conclusion

We conclude that extended genotyping permits an expansion of HPV testing to include all elements of risk management: (i) the detection of prevalent CIN3+ disease; (ii) estimating the 3-year risk for future CIN3+; and (iii) permitting unambiguous detection of persistent infections as another indicator of clinical risk.
LONG TERM SCREENING PERFORMANCE OF CYTOLOGY, HPV 16/18 GENOTYPING, AND E6 ONCOPROTEIN IN TRIAGING WOMEN WITH POSITIVE HIGH-RISK HPV TEST IN CHINA

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Background / Objectives

HPV genotyping and cytology test have been recommended for the triage of HPV positive women to colposcopy. E6 oncoprotein is a necessary agent of HPV driven oncogenic transformation as a potential biomarker in triaging HPV positive women. However the clinical performances of HPV genotyping, cytology and E6 oncoprotein test are almost based on cross-sectional studies and no data from prospective cohort are reported in Chinese women. This study was aimed to evaluate the long-term role of cytology, type-specific HPV and E6 oncoprotein triage based on the population-based cervical cancer screening cohort in mainland China.

Methods

We analyzed the cohort database of Shanxi Provincial of Cervical Cancer Screening Study from 2005-2014, 1734 women aged 45–55 were screened by the Hybrid Capture 2 (HC2), liquid based cytology (LBC) tests with experienced cytologist and visual inspection with acetic acid (VIA), and referred to colposcopy and biopsy if any test was positive. HPV16/18 E6 oncoprotein (E6) testing was performed on cervical samples with positive HC2 results. They were followed up in 2010 and 2014 with HPV testing, LBC and VIA (except in 2014). Based on screening results in 2005, cross-sectional and prospective clinical performance by visit, with 5-year and 10-year screening performance of CIN2+ were calculated.

Results

Among 290 HR-HPV positive women in 2005, incident sensitivity detecting CIN2+ for all triage methods decreased while incident specificity increased over time. During
the 10-year follow-up, cytology with ASC-US cut-off had the highest incident sensitivity (95.2%, 92.2% and 91.5% in 2005, 2010 and 2014, respectively), HPV16/18 E6 protein testing had the highest incident specificity (92%, 94.5% and 94.7% in 2005, 2010 and 2014, respectively), and HPV16/18 genotyping were at intermediate efficiency (sensitivity were 71.4%, 68.6% and 63.3%, and specificity were 70.6%, 74.6% and 74.9% in 2005, 2010 and 2014, respectively); positive predictive value of CIN2+ in cytology with ASC-US cut-off, HPV16/18 genotyping, and HPV16/18 E6 protein testing were 96.5%, 88.5%, and 83.3% in 2014, and negative predictive value were 38.8%, 40.0%, and 58.6%, respectively.

Conclusion

Genotyping for HPV16/18 could be recommended in triaging HPV-positive women while in situations without high quality cytologic screening. Positive HPV16/18 E6 protein testing might be a good potential biomarker for triage with its high predictive value of the long-term risk of CIN2+. 
FC 17-11
COMPARATIVE PERFORMANCE EVALUATION OF SCREENING TOOLS FOR POINT OF CARE CERVICAL CANCER SCREENING AND PRE CANCER TREATMENT AMONG WOMEN LIVING WITH HIV : CASE FOR INTEGRATING CERVICAL CANCER SCREENING WITH HIV TESTING AND COUNSELING CENTERS IN RESOURCE LIMITED SETTINGS

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Background / Objectives

Human Immunodeficiency Virus (HIV) related immunosuppression predisposes co-infection with Human Papillomavirus (HPV) and increases the risk of cervical intraepithelial neoplasia (CIN) and cervical cancer. Centers for Disease Control (CDC) has included invasive cervical carcinoma among the AIDS-defining conditions. India shares 25% of global burden of cervical cancer and a third large burden of HIV-infected women [1,2] who currently continue to live longer due to improved access to antiretroviral therapy. A cross-sectional study was performed to determine the prevalence of HPV infection, risk of cervical pre-cancer and clinical performance validation of Visual Inspection with Acetic Acid (VIA), Pap cytology and HPV tests among women living with HIV/AIDS in Mumbai, India.

Methods

309 HIV Positive women attending cervical cancer screening services in a tertiary care centre between 2009-12 were enrolled for cervical cancer screening using Visual inspection with acetic acid (VIA), Pap cytology and HPV testing. Total 291 HIV Positive women were included in the analysis. 18 were excluded from analysis for incomplete investigations. Screen positive women by either of the screening tests were subjected to histopathology confirmation with colposcopy guided cervical punch biopsy.

Results

Screen positivity rate for cervical cancer screening by VIA , HPV DNA and Conventional Cytology test was 36.1% (105), 32.3% (94) and 16.4% (48) respectively. Using a Histopathology CIN 2+ threshold, the sensitivity of VIA, HPV DNA and Conventional cytology tests were 0.96 (95% CI: 0.78 - 1.00), 0.91 (95% CI: 0.71 - 0.99), 0.64 (95% CI: 0.41 - 0.83) and specificities were 0.70 (95% CI: 0.64 - 0.76), 0.71 (95% CI: 0.65 - 0.76), 0.98 (95% CI: 0.95 - 0.99) respectively.

Conclusion
The prevalence of HPV infection and CIN are significantly higher in the HIV-positive women in India. VIA performed equivalently to currently approved but expensive HPV DNA tests and was highly sensitive compared to conventional cytology. HPV DNA and cytology are currently not feasible in low resource settings due to its logistics, financial and technical requirements. It is feasible to integrate low cost VIA as a point of care cervix cancer screening modality that facilitate “See and Treat” approaches, which would improve compliance to pre cancer treatment with the current HIV testing and counseling centers in the country.

References


A new technique of DNA isothermal amplification techniques in cervical cancer screening

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**Background / Objectives**

**Background**: Nucleic acids isothermal amplification techniques are gaining a wide popularity as diagnostic tools due to their simple operation, rapid reaction and without using thermocycler machine, e.g. Aptima HPV E6&7 mRNA test (HOLOGIC). However, mRNA based test asks for strict specimens preservation which limits its use in low resource setting. Isomega HPV test kit (National Bio-Founder, China) is a new HPV DNA based on isothermal amplification technology that can detect 15 high risk HPVs with simultaneous genotyping of HPV16 and HPV18 in a single tube by real-time fluorescence. The test procedure is simple and less than 90 minutes for operation on low cost and simple equipment. **Objectives**: To evaluate the agreement of HPV detection between Isomega HPV test and cobas 4800 HPV test (Roche) and their performance for cervical cancer screening.

**Methods**

**Methods**: Isomega HPV test and cobas 4800 HPV test were performed on cervical specimens collected from 2,774 women aged from 30 to 64 in high risk areas of cervical cancer in China. The sensitivity and specificity in detection of CIN2+, and the agreement between Isomega HPV test and cobas 4800 HPV test were assessed.

**Results**

**Results**: The positivity rate of HPV 16 and 18 and other HR-HPV were 3.64%, 1.19%, 13.95% for Isomega HPV test and 3.53%, 1.29%, 13.63% for cobas 4800, respectively. The agreement between two methods was 94.77% (Kappa=0.818) for HR-HPV types, 99.60% (Kappa=0.943) for HPV16, 99.78% (Kappa=0.868) for HPV18 and 93.62% (Kappa=0.744) for other high-risk types. The sensitivity and specificity were 87.76%, 82.86% for Isomega, and 89.80%, 85.06% for cobas 4800.

**Conclusion**

**Conclusions**: Isomega HPV test showed high accordance with cobas 4800 HPV test with good sensitivity and specificity, would be a cost-efficient, fast and reliable HPV assay for cervical cancer screening in low resource setting.
Effectiveness of HPV testing in ASC-US to predict HSIL

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Background / Objectives

Cervical cancer screening management is changing drastically since the introduction of human papillomavirus (HPV) tests. HPV testing in ASC-US has been shown to improve the management of ASC-US by a better risk stratification of HSIL+ risk. We aimed to evaluate the accumulative risk at 5 years of HSIL+ at first screening visit for cervical cancer with a particular focus on ASC-US and HPV detection in Catalonia, Spain.

Methods

The study included 169358 women aged 25-65 living in Catalonia (Spain) participating for the first time in the opportunistic screening during 2010-11 at the NHS facilities and follow up until December 2015 for any further cervical cytology record. Kaplan Meier and Cox methods were used to estimate the time to HSIL+ and the hazard ratio of HSIL+.

Results

Women with ASC-US having a negative HPV had a similar HR than those having a negative cytology. While women with ASC-US and a positive HPV test had a similar HR of HSIL+ as that seen for LSIL cases (HR=29.4 and HR=25.5 respectively). Women ASC-US with unknown HPV data had an intermediate HSIL+ risk (HR=15.09).

For the first time in Spain we show the robustness of HPV testing in the prediction of HSIL+ lesions in women with a diagnosis of ASC-US at the primary health level.

A literature review on the topic will be presented.

Conclusion

In agreement with other settings we show the robustness of HPV testing in the prediction of HSIL+ lesions in women with a diagnosis of ASC-US at the primary health level in Spain.
Colposcopic and histopathologic evaluation in women aged 56-64 with HPV-persistence 1 and 3 years, respectively, from the organized primary HPV screening in Sweden.

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Background / Objectives

To evaluate the colposcopic and histopathologic findings in HPV++ women 56-64 years in an organized primary HPV screening program.

Methods

The organized screening program in Stockholm county randomised all resident women 56-60 years to either primary HPV screening with cytology triage or to primary cytology and HPV triaging for LSIL. In HPV screening, HPV+/Cyt-women had a repeat HPV test 1 year later. The repeat HPV test was changed to three years later in May 2013. Attendance rates were similar with the 2 policies. (HPV screen: 7325 women and cytology screen: 7438 women). All HPV++/Cyt- women were invited to colposcopy after 1 year, and HPV++ cyt-/+after 3 years, performed by the same expert gynecologist.

Results

After 1 year 52% (87/167) were HPV negative or declined participation. 80 women had a colposcopy. 74% were persistent for the same HPV type (59/80), the most common type HPV 16(27%). 51% (41/80) had atrophic epithelium. 40% (32/80) a transformation zone (TZ) type 3. 45% (36/80) had a colposcopic lesion, 15% (12/80) abnormal cytology from the endocervix and 24% (19/80) CIN2+ in the cervical biopsy or conisation following the colposcopy. Of the 16 women with HPV 16 persistence at the second visit 6 developed HSIL.

After 3 years, 58% (77/132) were HPV neg or declined participation. So far 36/55 HPV++women have had a colposcopy and results are available for 18 patients 9 who were HPV++ Cyt-. 8/9 had atrophic epithelium, 6/9 a transformation zone (TZ) type 3, 3/9 had a colposcopic lesion, 4/9 abnormal cytology from the endocervix and 0/9 HSIL in the random cervical biopsy, (no cone biopsies included yet).
Out of 9 patients who were HPV++ and Cyt+ (7/9) had atrophic epithelium, (3/9) a transformation zone (TZ) type 3, (7/9) had a colposcopic lesion, (6/9) abnormal cytology from the endocervix and (2/9) HSIL in the cervical biopsy. More results will be presented.

Conclusion

Women were not aware of their randomization however similar attendance rates was noted in both arms. Colposcopic evaluation of cytologically negative, HPV ++ positive women resulted in a PPV for HSIL in histopathology of 24% (19/80) after 1 year. PPV for HSIL in case of HPV 16 persistence was 38% after 1 year. Results after 3 years will be presented.

Atrophy and TZ 3 are challenges while blind biopsies or diagnostic cone biopsies, HPV genotyping and cytology triage including endocervical sampling are options for this group of women.
EVIDENCE FOR CLINICAL APPLICATION OF EXTENDED HPV GENOTYPING IN PERSISTENCE TRACKING AND TEST-OF-CURE: A SYSTEMATIC REVIEW

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Background / Objectives

Management guideline originators have not yet included an analysis of the body of science published during the last decade about the clinical value of extended HPV genotyping (xGT) in follow-up of women with abnormal results and in test-of-cure. This targeted systematic review addresses key questions (KQ) that pertain to the effectiveness of including xGT results with follow-up testing for reducing cervical cancer mortality and incidence. KQ1 evaluates xGT for cumulative risk estimates in the scenario of persistence. KQ2 evaluates xGT for risk discrimination in the follow-up of women with prior abnormal results. KQ3 evaluates xGT in test-of-cure of women with prior treatment of cervical intraepithelial neoplasia (CIN)2 or CIN3.

Methods

We searched the Database of Abstracts of Reviews of Effects, the Cochrane Database of Systematic Reviews, PubMed, and the Health Technology Assessment database from 2000 through 2017 for relevant controlled trials and observational studies. We supplemented by hand-searching of retrieved article reference lists. Eligible studies included prospective studies of women and retrospective studies of residual specimens from women that were screened or tested using human papillomavirus DNA tests. The reference standard was cervical intraepithelial neoplasia 2 (CIN2) or CIN3 or CIN2+ or CIN3+ or invasive cervical cancer (squamous and/or adenocarcinoma). The timeframe was 1-year, or 3-year, or 5-year, or greater than 5-year for persistence tracking. The timeframe was 6-month, 1-year, 18-month, or 2-year for follow-up and test-of-cure. Relevance screening, data extraction, risk of bias analyses, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the QUADAS evidence-based quality assessment tool of diagnostic accuracy studies, supplemented by the STARD checklist and GRADE methodology.

Conclusion
The available evidence supports the conclusion that reporting xGT results supports clinical decision making for follow-up of women by discrimination of risk. Based on large studies, xGT appears very promising as follow-up of persistence versus clearance, to discriminate risk and support risk-based clinical action steps by the principle of equal management for equal risk. Models for different management paradigms are described. The information in this report is intended to help guideline panels, policymakers, clinicians, and women make informed decisions about the selection of health care services, is intended as a reference, and not as a substitute for clinical judgment.
RISK OF CERVICAL CANCER AFTER ATYPICAL GLANDULAR CELLS FOUND AT SCREENING IN THE NETHERLANDS

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Background / Objectives

Atypical glandular cells (AGC) abnormalities are less common squamous cell abnormalities in cytology, but can be indicative a number of conditions that vary in severity. Understanding the risk of invasive cervical cancer after AGC are found at screening is important for management of these patients. In this study, we examined the risk of cervical cancer in the next primary screening round after AGC compared to the risk after normal cytology, low-grade squamous intraepithelial lesion/atypical squamous cells of undetermined significance (LSIL/AS-CUS) or high-grade squamous intraepithelial lesion (HSIL) abnormalities.

Methods

Using data from the Dutch Pathology database (PALGA) from 1 January 1996 to 31 March 2014, we conducted preliminary analysis on all primary screening rounds that had cytology results with AGC only, LSIL/AS-CUS only, HSIL only or normal cytology and investigated the result of the next primary screening round. Screening rounds with both squamous abnormalities and AGC cells, or with cancers diagnosed within the same screening round as the abnormality were excluded from analysis. All cervical cancer types were included. Crude rates of cancer diagnosis in the next screening round were calculated for each result category. Follow-up time was calculated from each date of primary smear until either a) the next primary screening round, b) a primary histology diagnosis, c) 8.5 years of follow-up or c) 31st March 2014, whichever came first. Hazard ratios (HR) were calculated using SAS 9.4.

Results

From 9,659,626 cytology smears included in our analysis, 3,156 cervical cancers were identified in the next screening round: 3,037 after normal cytology, 29 after AGC only, 29 after LSIL/AS-CUS only and 61 after HSIL only cytology. Crude rates per 100,000 women-years at risk were highest for the AGC only group (38.8 per 100,000) compared to other groups (normal: 8.7 per 100,000; LSIL/AS-CUS: 12.5 per 100,00; HSIL: 24.6 per 100,000). Compared to normal cytology, the HR was significantly higher for AGC only smears (4.3, 95% CI: 3.0, 6.2), with lower HRs for both HSIL only smears (2.9, 95% CI: 2.2, 3.7) and LSIL/AS-CUS smears (non-significant - 1.4, 95% CI: 1.0, 2.0).

Conclusion
Preliminary results indicate that women with AGC only abnormalities found on cytology screening have a higher risk of a cancer diagnosis at the next screening round compared to women with a squamous cell abnormality. This could be caused by false-negative follow up tests or suboptimal management of these women. Given that with primary HPV screening relatively more women with an AGC smear will be found, gynaecologists need to be aware of this increased cancer risk.
RISK FACTOR ANALYSIS OF RESIDUAL HSIL AFTER LEEP: A CLINICAL STUDY OF 1511 LEEP CASES AT OB&GY HOSPITAL OF FUDAN UNIVERSITY

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Background / Objectives

The purpose of this study was to analyze risk factors of residual cervical high grade squamous intraepithelial lesion (HSIL) after loop electrosurgical excision procedure (LEEP).

Methods

This retrospective study was carried out on 1511 patients with HSIL who underwent LEEP from January 2011 to December 2013 at Obstetrics and Gynecology Hospital of Fudan University. All patients were followed 3-6 months after the LEEP with ThinPrep cytologic test (TCT), HR-HPV test and colposcopy guided biopsy.

Results

Among the 1511 HSIL cases, 57(3.8%) cases suffered residual HSIL or more serious lesion, including 48 cases with HSIL, 6 cases with invasive squamous cell cancer, 3 cases with adenocarcinoma. Residual rate are different among different age groups. The residual rate was 2.8% (4/143) in the age below 30 group, 3.0% (36/1202) in the age 30-49 group, 10.2% (17/166) in the age beyond 50 group. The residual rate of LEEP positive margin was obviously higher than the negative margin group (7.8% vs 2.9%). Cytologic abnormality showed higher residual than non-residual with significant difference (6.0% vs 1.7%). Patients with positive postoperative hrHPV had a higher residual rate than the negative group (1.4% (10/730) vs 3.4% (7/207)), while there was no statistical significance. There was statistical significance on perimeter and thickness between residual group and non-residual group (2.6±0.8 cm VS 2.9±0.7 cm, 0.6±0.3 cm VS 0.7±0.2 cm (P<0.05)), though depth showed no obvious difference (1.4±0.5 cm vs 1.5±0.3 cm, P>0.05). Different positive margin has diverse residual rate, with endocervical positive margin was 17.5% (10/57), positive margin undetermined was 8.2% (8/97), which is higher than margin negative group (2.9%, 36/1242). Ectocervical margin and deep margin positive showed no difference with margin negative ones. Multivariate logistic analysis showed that age (OR=2.9, P<0.05) and postoperative cytology follow-up positive (OR=3.0, P<0.05) were independent risk factors of residual lesion.

Conclusion
Aging, Endocervical positive margin and abnormal cytology follow-up were high risk factors that lead to postoperative residual lesion. Endocervical positive margin, positive margin undetermined were easier to suffer residual lesion than margin negative and ectocervical positive margin.
FC 18-06
HPV CELL-FREE DNA IN PLASMA AS AN USEFUL MARKER FOR MONITORING RELAPSE OF CERVICAL CANCER

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Background / Objectives

Patients with CC have a relapse rate ranging from 8% to 49%. Within two years of follow up, 62% to 89% of relapses are detected. Nowadays, the tests used to detect recurrence are imaging and cytopathology from the vaginal vault, but there are no available specific tests yet. Cell-free circulating DNA (cf-DNA) is a non-invasive biomarker easily obtained from plasma and serum. Several studies have shown the possibility to detect and quantify nucleic acids in the plasma of cancer patients and that the changes in cf-DNA potentially reflect the changes that occur during tumorigenesis. This non-invasive diagnostic tool may be useful in screening, prognosis and monitoring response to treatment. Therefore, the development and standardization of non-invasive laboratory tests that are able to identify tumour markers and to make early diagnosis of the disease recurrence increase the chance of cure by appropriate treatments. Objective: This study aimed to detect HPV DNA in the plasma of patients with CC, to assess its potential utility as an early marker of recurrence.

Methods

A tumour biopsy and a blood sample of patients with CC, attended in ICESP and HC Barretos, were collected before and after CC treatment. HPV genotyping was performed on the DNA obtained from the tumour. cfDNA was extracted from plasma samples obtained pre and post-treatment and submitted to type-specific real-time PCR spanning the E6 region from HPV 16 and 18. Patients were followed for at least 2 years after treatment and plasma samples obtained at least once during this period.

Results

137 patients entered the study, 120 bearing HPV-16 positive cancers (87.6%), 12 HPV-18 (8.8%) and five harboured both HPV -16 and 18 DNA (3.6%). The presence of HPV DNA in pre-treatment plasma was observed in 58.8% (77/131) with viral load ranging from 204 copies / ml to 2,500,000 copies / mL. HPV DNA frequency in pre-treatment plasmas increased with advancing clinical tumour stage: I - 45.2%, II - 52.5%, III - 80.0% IV - 76.9%, (p = 0.0189). The presence of HPV DNA in the post-treatment plasma was detected in 27.3% (30/110). The average time of relapse was
3.1 years (2.7 to 3.5 years). HPV DNA was evidenced up to 460 days before clinical diagnosis of recurrence. Patients who had the presence of HPV DNA in plasma after treatment had a worse prognosis compared to those who were negative, strongly correlating to poor survival rates and shorter disease-free time intervals.

Conclusion

In patients with CC, HPV-DNA in the plasma can be a useful early marker for monitoring relapse and progression of the disease.
PREVALENCE AND RISK FACTORS FOR MULTIZONAL NEOPLASIA IN A COHORT OF HIGH-RISK WOMEN


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Background / Objectives

Multizonal Anogenital Neoplasia is defined as the presence of high-grade squamous intraepithelial lesion (HSIL) or carcinoma concurrently in two or more of the following sites or zones: Perianus, Anal canal, Vulva, Vagina or Cervix. In each zone, disease may be uni- or multifocal. Homerton Anal Neoplasia Service (HANS) is a tertiary referral centre, receiving referrals from across the UK. It screens women at risk of HPV related dysplasia or carcinoma with a multizonal assessment and provides treatment of HSIL. Multizonal assessment is a thorough examination of the genital tract, performed with the woman in lithotomy position with 5% acetic acid applied to the cervix, vagina, vulva, perianus and anal canal, which is then visualised using colposcope as a microscopic aid. Biopsies are taken of suspicious areas.

Aims: Identify disease prevalence in the anogenital region in a cohort of women at high-risk of multizonal neoplasia

Methods

Retrospective case note review of one hundred women referred to the HANS since January 2011 for multizonal assessment.

Results

Women were referred to the HANS Unit for multiple reasons, most commonly for known persistent vulval HSIL (n=19) and widespread anogenital dysplasia (n=17). Mean age was 49 years (range 26-85 years) with median follow up of 24 months. Half the women (50%) were immunosuppressed. Twenty six women had previous vulvectomy for HSIL, 13 women had a hysterectomy for cervical HSIL (CIN) and 25 women had a previous anogenital cancer. All women underwent a multizonal assessment and 42 women were found to have two or more zones of high grade dysplasia. Sixteen women with no perianal disease had anal canal HSIL, one of which had cancer. Over one quarter of women had a new zone of HSIL diagnosed (n=27). Six occult cancers were found at first appointment and two women progressed to cancer over follow up, one had a severe immune defect and the other woman was likely to have had an occult cancer at initial presentation.

The risk factors for multizonal neoplasia were analysed. Twenty seven women (64.3%) were immune suppressed in the multizonal neoplasia group (42) compared to twenty three (39.7%) of those without multizonal neoplasia (58). A history of
previous anogenital cancer was more common in the multizonal neoplasia group (38.1%) than in the women without multizonal disease (15.5%).

**Conclusion**

The absence of perianal disease does not preclude the presence of anal HSIL or occult cancer. Concentrating only on the referral zone would miss HSIL disease in other related anogenital tract sites. Multizonal assessment is essential to diagnose occult areas of HSIL or carcinoma in high risk women.
FC 18-08
Is p16/ki67 dual-stained cytology essential at a colposcopy department?

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Background / Objectives

The main decrease of cervical cancer mortality and incidence was provided by cytology-based screening programs. The interpretation of cytology is pathologist-dependent, and due to that some countries shift screening to human papillomavirus (HPV) DNA detection. HPV test has higher sensitivity and higher negative predictive value for detection of cervical intraepithelial neoplasia (CIN). Nevertheless, HPV DNA testing has a lower positive predictive value, because most high-risk infection clears spontaneously. Recently, a widely-studied biomarker, p16/Ki-67, demonstrates to be useful on limiting the unnecessary referrals to colposcopy units. This property is based on the fact that simultaneous presence of p16/Ki-67 indicates deregulation of cell-cycle, mediated by transforming high-risk HPV infections.

The main goal of the study was to evaluate the utility of p16/Ki-67 dual-stained cytology, for identification of CIN in high-risk HPV-positive women, previously referred to a colposcopy unit.

Methods

It was performed a prospective cohort study of 46 high-risk HPV-positive women followed at a colposcopy unit. They were evaluated by p16/Ki-67 dual-stained cytology from February 2016 to March 2017. Statistical analysis was performed by STATA 13.1 program.

Results

The women included at the study, had a median follow-up time at the unit of three years (p5:6 months; p95:5 years) and a median age of 44 years (p5:29; p95:62). Of high-risk HPV-positive women, thirty-three (72%) had negative (NILM) cytology, ten (22%) had low-grade squamous intraepithelial lesion (LSIL) and three (6%) abnormal squamous cells of undetermined significance (ASC-US). Positive p16/Ki-67 was identified in twelve women with NILM cytology, in six with LSIL and in one with ASC-US cytology. The positive predictive value to CIN was 81.8% (95%CI:48.2-97.7) with a sensitivity of 90.0% (95%CI:55.5-99.7).

At the same sample, liquid-based cytology (ThinPrep©) had a sensitivity of 60.0% and specificity of 89.5%, HPV test (Cobas©) had a sensitivity of 91.7%, specificity of 20.7% and a positive predictive value of 32.3%. Colposcopy had a sensitivity of 87.5% and a positive predictive value of 80.8.
From positive p16/ki67 dual-stained cytology, 58% women were submitted to expectant treatment and 42% to invasive one. All women maintain medical surveillance at the unit.

**Conclusion**

The p16/Ki-67 dual-stained cytology demonstrates high sensitivity and positive predictive value to intraepithelial neoplasia however, it will be needed a larger sample and a higher time of follow-up to generate solid conclusions.
FC 18-09
CLINICAL-PATHOLOGICAL VARIABLES ASSOCIATED WITH CERVICAL CONIZATIONS SPECIMENS WITHOUT HIGH-GRADE INTRAEPITHELIAL LESION: A STUDY OF 221 CASES.

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Background / Objectives

Comparison of clinical-pathological variables in a series of cervical conizations associated with the presence or absence of high-grade intraepithelial lesion or carcinoma.

Estimation of the utility of the immunohistochemical staining for p16 to identify conization specimens without intraepithelial lesion or carcinoma.

Methods

We reviewed our conizations from 2010 to 2016. In every cases without ≥ CIN 2 ("negative cone"), 3 hematoxylin and one p16 slides were added. Former colposcopy biopsies were reassessed.

The clinical variables were: age, smoking, spreading of the colposcopic lesions, number of colposcopic biopsies, vaccination status against HPV prior to conization.

The collected pathological features were: former cytology result; HPV status and colposcopic biopsy diagnosis prior to conization; lesion length and intensity of the inflammatory infiltration in the colposcopic biopsy; initial and final diagnosis in negative cones after morphological reassessment and hematoxylin and p16 additional slides.

Pap smears were performed in liquid medium. HPV determination was performed with COBAS® 4800 HPV Test

Results

There were 221 conizations, with a mean age of 38 years and a 50.8% of smokers. In a 71.9% of the cases, the previous cytology was ≥ASCUS. In a 99.1% of women, HPV status was positive, being a 54.5% of them positive to HPV16 and 48.4% to neither types 16 or 18.
There were a 59.5% of the lesions affecting an unique quadrant, with a 60.3% of single biopsies. A 55.9% of the women were vaccinared between the colposcopic biopsy and conization. A 97.1% of the colposcopic biopsies were ≥ CIN 2, with an average length of 3.37 mm. A 35.3% of such biopsies showed moderate to intense inflammatory infiltration.

Initially there were 44 (19.90%) "negative cones", which was shorten to 27 (12.21%) after morphological reappraisal and hematoxylin and p16 addition, being a 38.63% reduction. 4 "negative cones" were retrieved due to the morphological review, another 12 by using p-16 and, finally, one case was reclassified as low grade intraepithelial lesion.

Comparing clinical and pathological items we only found a statistical trend to younger age and lower lesion spreading in the "negative cones" and a statistical significance in favour to a larger size of colposcopic lesions in pathological cones.

**Conclusion**

In our series we can hardly see statistically significant differences between clinical-pathological variables of the pathological cones and the negative cone.

Before establishing a diagnosis of negative cone we should completely reassess the case and to perform immunohistochemical study for p16.

**References**

1. Examination of sources of diagnostic error leading to cervical cone biopsies with no evidence of dysplasia. Alison Carrigg, DO Crystal Teschendorf, CT (ASCP), Deidre Amaro. Am J Clin Pathol 2013;139:422-427

COMPARISON OF PAIN CONTROL BY LIDOCAINE SPRAY AND PARACERVICAL BLOCK DURING LOOP ELECTROSURGICAL PROCEDURE: A RANDOMIZED CONTROL TRIAL

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Background / Objectives

Cervical cancer is second most common new case and third most common cause of death of cancer patient in developing country. The loop electrosurgical excision procedure (LEEP) is worldwide used for diagnosis and treatment in CIN2-3, precancerous lesion since 1990. The anesthetic technique common use during LEEP are paracervical block and submucosal block. The paracervical has some risk for local anesthetic systemic toxicity. Açmaz et al report adverse effect from paracervical block 2 patients have bradycardia. The new anesthetic technique lidocaine spray has report that effective use during endoscopy, intubation, fractional and curettage, suctional and curettage, and LEEP.

This study is compare effective of pain control between paracervical block and lidocaine spray and adverse effect.

Methods

A randomized control trial was conducted in 132 women who underwent LEEP of cervix. The participants were randomly allocated to two groups. PB group were anesthetized by standard paracervical block using 10 mL of 2% lidocaine with 1:100,000 of epinephrine at 4 and 8 o’clock locations. LS group were locally anesthetized by four puffs (40 mg) of 10% lidocaine spray applied thoroughly to the cervix. The pain score at during anesthesia, during excision and 30 minutes post excision were compared.

Results

A total of 132 LEEPs were performed with 66 participants in LS group and 66 participants in PB group. The age, parity, menopausal status, indications and tissue specimen volume were not significant difference in both groups. The mean pain scores during excision were 5.2±0.3 in LS group and 4.1±0.4 in PB group (mean difference 1.1, 95%CI 0.8-2.1, p value=0.03). The pain score during anesthetized was significant lower in LS group (2.0±0.3 vs 3.1±0.3, mean difference 1.1, 95%CI 0.3-1.9, p value=0.008). There had no adverse effect in LS group compare with 8 cases in PB group (tinnitus, numbness and tachycardia with hypertension).

Conclusion
The pain score in local 10% Lidocaine spray group was significant higher from standard paracervical block during the LEEP procedure of cervix but had less adverse effect.

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References


CROSSWALKING EUROPEAN GUIDELINES ON THE MANAGEMENT OF VAGINAL DISCHARGE AND THE MANAGEMENT OF STI

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Background / Objectives

Screening guideline originators (WHO, IUSTI, CDC, country-specific) have not included STI testing within the contextual vaginal discharge or vaginitis guidelines and have not included vaginitis testing within the contextual STI screening and management guidelines. Women may present with a request for STI screening and have vaginitis, with or without an STI. Women may present with a complaint of abnormal vaginal discharge and meet criteria for STI testing, but this is not explicitly stated in vaginitis guidelines.

Methods

We searched PubMed, Guideline International Network, WHO, IUSTI, CDC, and the Worldwide Web from 2010 through 2017 for relevant guidelines. We supplemented by hand-searching of retrieved guideline reference lists. Eligible guidelines publications included screening for STIs in women and/or included diagnostic guidance for women presenting with abnormal vaginal discharge or symptoms of vaginitis or symptoms of vaginosis. Relevance screening, data extraction, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the AGREE reporting checklist, RIGHT reporting checklist, and the Standards for Developing Trustworthy Clinical Practice Guidelines.

Conclusion

The review supports the conclusion that clinical practice guidelines about STI screening and management do not provide guidance for concurrent diagnosis and management of vaginitis or vaginosis. Similarly, clinical practice guidelines about diagnosis and management of abnormal vaginal discharge or symptoms of vaginitis do not provide guidance for concurrent screening and diagnosis of STIs. Guideline panels could choose to add content to their specific guideline, or could choose to add linkages to relevant guidelines. Crosswalking of European guidelines are tabulated. The clinical criteria for STI screening that should be checked during a problem visit for vaginitis are provided. The clinical criteria for vaginal discharge assessment for vaginitis that should be checked during a visit for STI screening are provided. The information in this report is intended to help guideline panels, policymakers,
clinicians, and women make informed decisions about the selection of screening and diagnostic services, is intended as a reference, and not as a substitute for clinical judgment.
RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) IKNIFE AND ITS CLINICAL APPLICATION IN THE TREATMENT OF CERVICAL ABNORMALITIES

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Background / Objectives

Cervical cancer and its precancerous form cervical intraepithelial neoplasia (CIN) commonly affect women of reproductive age. Fertility-preserving trachelectomy procedures are available, but if the excisional margins are not cancer-free, as is the case in 33% of procedures, these women must undergo a hysterectomy, therefore losing their child-bearing potential. Rapid Evaporative Ionization Mass Spectrometry (REIMS) analyzes electrosurgery-generated aerosols, using time-of-flight mass spectrometry to provide real time tissue identification without the need for sample preparation, raising the potential for use as an intraoperative diagnostic technique and improving the surgical and fertility outcome for one third of the women who undergo trachelectomy. We conducted a pilot study showing that REIMS can differentiate between cancerous and healthy cervical tissue thus presenting an innovative technique that could drastically improve fertility-sparing operations.

Methods

Cervical biopsies of 66 women were cut using a Covidien diathermy hand-piece. The surgical aerosol produced was transferred into a Waters Xevo G2-S mass spectrometer. The tissue samples were then stained for histopathological validation. These diagnoses were used in multivariate statistical analysis of mass spectroscopic spectral data, including principal components and linear discriminant analysis performed using Offline Model Builder software. Correct classification rate was checked using leave one patient out cross-validation.

Results

The study showed correct classification with REIMS of 91%, with correct identification of cancer tissue of 88.5% and of healthy tissue of 92.5%. Ongoing sample processing is currently being undertaken to investigate the correct classification rate with REIMS between the different grades of CIN.

Conclusion

Frozen section is the current method for intraoperative assessment of margin status at the time of trachelectomy, and the concordance between intraoperative frozen
section and final histology has been quoted as 84%, significantly lower than the preliminary results of REIMS. In addition to providing real-time information, thus reducing anaesthetic time, REIMS has the potential to improve the accuracy of intraoperative margin detection. This could potentially increase success rates of trachelectomy, leading to a truly advanced fertility sparing technique in modern surgery. This principle is also under investigation for its use in CIN to be ruled out into the colposcopy clinic.
AGE DISTRIBUTION AND PROBABILITY OF HYSTERECTOMY IN GERMANY

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Background / Objectives

Hysterectomy is the most common gynecological surgery in many industrialized countries. In Germany, the hysterectomy rate is high in comparison to other European countries. The aim of this analysis was to determine the distribution of age at hysterectomy as well as the age-specific probability of undergoing a hysterectomy between the ages of 0-64 in the German female population.

Methods

Analyses were based on data from the MARZY study, a prospective, randomized, population-based cohort study investigating early detection of cervical cancer in western Germany. At baseline, 6 429 women were invited to attend cervical cancer screening. The distribution of age at hysterectomy as well as indications for hysterectomy were reported. Based on survival analysis, which accounts for censoring at the age of interview, and the inverse probability weighting (IPW) method, the age-specific probability of undergoing a hysterectomy was estimated. The IPW method corrected for missing date of hysterectomy. Simulated calendar-period specific survival curves (1939-1979, 1980-1989, 1990-1999, 2000-2006) were computed to show how age and calendar year determine the probability of undergoing a hysterectomy.

Results

Data on hysterectomy were available for 4 719 women. Of these, 961 women (20.4%) had undergone a hysterectomy. The main indication for hysterectomy was uterine fibroids (48%). A total of 850 women (88.4%) reported a date when their hysterectomy had been performed. The highest proportion of women were hysterectomized between the ages of 40-44 (24.6%). The IPW corrected probability of having a hysterectomy between the ages of 0-64 was 0.354. The age-specific probability of hysterectomy was highest in the 45-49 year age group (0.078). The age-specific probability of hysterectomy decreased between the years 1939 to 2006.

Conclusion
Data from the MARZY study allowed valuable conclusions to be drawn about the distribution of age at hysterectomy as well as the age-specific probability of undergoing a hysterectomy in Germany.
Type-specific Human Papillomavirus DNA Load in Association with Prospective Risk of Cervical Intraepithelial Neoplasia: A Useful Triage Tool

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Background / Objectives

The ASCCP guidelines recommend referring non-16/18 high-risk human papillomavirus (HR-HPV) positive women with cytology ≥ASCUS (ASCUS+) to colposcopy. In low-resource areas without cytology, other screening methods are needed to triage HPV positive women. Using direct prospective evidence, our study compared the risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in non-16/18 HR-HPV and HPV 16/18 positive women stratified by viral load versus cytology, and evaluated the risk of CIN2+ by type-specific HPV viral load.

Methods

Using China's SPOCCS1 cervical cancer screening cohort, where 1,742 women were screened by liquid-based cervical cytology and Hybrid Capture 2 (HC2) at five-year intervals from 2005 to 2014, all HC2 positive samples were genotyped. Semi-quantitative viral load was measured by HC2 relative light units, with categories of low (1-9.99), moderate (10-99.99), and high (≥100) viral loads. Kaplan-Meier methods were used to estimate the ten-year cumulative incidence rate (CIR) of CIN2+ among pooled and type-specific HR-HPV women with baseline low, moderate and high viral loads, or ASCUS+.

Results

Among the 209 HR-HPV positive women at baseline, the highest CIR of CIN2+ were for women infected with HPV 16/18 (N=38/57), HPV 16 (N=36/57), non-16/18 HR-HPV (N=19/57), HPV 31(N=7/57), and HPV 58 (N=7/57). Any HR-HPV, HPV 16/18, non-16/18 HR-HPV or HPV 16 positive women had significantly higher CIRs of CIN2+ at moderate or high viral loads than at low viral loads. Similar trends were observed for HPV 18, 31, 33, 52, and 58 positive women, but limited sample sizes prevented reaching statistical significance. For HPV16/18 women, the CIR of CIN2+ with ASCUS+ was 55.8% (95%CI: 42.2-67.4%) and CIRs with low, moderate, and high viral loads were, respectively, 25.7% (95%CI: 9.3- 46%), 43.2% (95%CI: 26-59.3), and 62.6% (95%CI: 40.6-75.5%). For non-16/18 HR-HPV women, the CIRs of CIN2+ at low and moderate viral loads were, respectively, 5.1% (95%CI: 0.9-15.2%)
and 18.2% (95%CI: 21.6-54.9). The CIR of CIN2+ for non-16/18 HR-HPV positive women with ASCUS+ was 34.6% (95%CI: 21.9-47.6%) and comparable to that of non-16/18 HR-HPV high viral load (37.8%, 95%CI: 21.3-54.3%) (P>0.05).

**Conclusion**

HPV16/18 and non-16/18 HR-HPV viral loads could predict the ten-year CIR of CIN2+. HPV16/18 positive women should be directly referred to colposcopy regardless of viral load, as those with even a low viral load had a notably high risk of CIN2+. For non-16/18 HR-HPV women, using a viral load cutoff of ≥100 RLU had a comparable CIR of CIN2+ compared to ASCUS+ and presents a valuable triage tool for non-16/18 HR-HPV women in areas where cytology is not readily available.
WHOLE-GENOME SEQUENCING ANALYSIS OF HPV18 DIVERSITY IN THE NETHERLANDS

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Background / Objectives

Whole-genome Sanger sequencing was used to identify HPV18 variant diversity in the population. In addition conservation of HPV18 infections over time and the occurrence of HPV18 type-specific (TS) reinfection events were assessed. SNPs occurring in clearing infections and persistent infections were compared to identify possible differences between groups.

Methods

Vaginal self-samples were collected annually in up to four rounds from women (16-29y) participating in the Chlamydia trachomatis Screening Implementation program in the Netherlands. HPV-DNA detection and genotyping was performed using the SPF10-DEIA-LiPA25 system. Persisting and clearing HPV16 infections were selected and subjected to Sanger WGS. Persisting infections were defined as HPV18 positive by genotyping at two subsequent sampling moments. Clearing infections were defined as HPV18 positive initially, with at least one HPV18 negative follow-up sample.

Results

Complete genome sequences were obtained from 51 study participants having 24 persistent and 27 clearing infections, resulting in a total of 52 unique HPV18 variants (including one HPV18 reinfection event). Persistent infections were completely conserved through time, with up to three years between the initial sample and the last follow-up sample. One reinfection event was identified, initially considered a persisting HPV18 infection, with unique HPV18 variants at both sampling moments. The identified variants predominantly clustered with sublineages A3 (31 variants) and A1 (11 variants). Other sublineages identified in this cohort are A4 (2 variants), A5 (1 variant), B1 (5 variants) and B2 (1 variant). Although the dataset size is limited, SNP comparison did not identify strongly acting nucleotide differences resulting in infections clearing or persisting.

Conclusion

A remarkably high HPV18 variant diversity was found in a Dutch cohort from sequencing 51 HPV18 infections. Lineages A1 and A3 are predominantly present in the Netherlands. Persistent infections are completely conserved through time with up to three years follow-up. One HPV18 variant reinfection event was identified, initially considered a persistent HPV18 infection by conventional genotyping.
NATIONWIDE AND COMPREHENSIVE HUMAN PAPILLOMAVIRUS GENOTYPING OF INVASIVE CERVICAL CANCERS

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Background / Objectives

The Swedish National Cervical Screening Registry collects and evaluates nationwide, comprehensive data as a basis for optimization of organized cervical cancer prevention. Most data are imported via exports from administrative databases at the healthcare providers, but the comprehensive biobanking system of all cases of cancer in the nation is also a carrier of population-based data that could be read and imported to the registry. As a proof-of-principle, we identified all cervical cancers diagnosed in Sweden during a 10 year period (2002-2011; 4254 confirmed cases), requested the archival blocks and subjected them to HPV genotyping.

Methods

The Swedish Cancer Registry was used to identify the cases and the diagnosing pathology lab. The diagnostic blocks were requested and sectioned at an accredited sectioning company. In between each case block, a blank block containing only paraffin was sectioned, as a control for contamination. The blank-block had to be negative in all tests and the case-block positive for beta-globin. Following DNA extraction, HPV genotype data were retrieved using beta-globin real-time PCR and HPV genotyping using modified general primer (MGP)-PCR (primer target L1) and Luminex. Confirmed invasive cancers that were “HPV-negative” by Luminex were also analysed by real-time PCR for HPV16 and HPV18 (primer target E7 and E6), and will be sequenced using Illumina technology.

Results

Although all pathology biobanks were - in principle - open access, not all agreed to participate. 2954/4254 (69%) cases were actually included and valid genotyping data could be obtained for 2852 cases (97% of included). The most common type was HPV16 (60% of HPV-positive, 50% of all valid cases), followed by HPV18 (19%/15%), HPV45 (8%/6%), HPV31 (3%/2%), HPV33 (2%), HPV52 (2%), HPV39 (1%), HPV70 (1%), HPV56 (1%), HPV35 (1%), HPV58 (1%) and HPV59 (1%). 96% of all HPV-positive tumours contained only one HPV type. Real-time PCR for E6/E7
performed only for “HPV negative” cases found that 12% of these were HPV16 positive and 7% were HPV18 positive.

**Conclusion**

Nationwide, comprehensive HPV genotyping of consecutive series of invasive cervical cancers is readily doable as part of the data importing tasks of a cervical screening registry, improving the possibilities to monitor the effectiveness of cervical cancer prevention and continued monitoring of the HPV-type specific disease burden.
HPV PREVALENCE AND RISK FACTORS ASSOCIATED WITH HIGH RISK TYPES IN A LOW INCOME POPULATION

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Background / Objectives

HPV is the most common sexually transmitted infection and is associated with cervical cancer in women – the fourth cause of mortality of female cancer in Brazil. Surveys on HPV prevalence and lifestyle factors relevant to HPV transmission are essential to monitoring the infection and plan prevention programs in low income countries to fight against HPV related cancers. Therefore, we aim to estimate the prevalence of genital HPV in women and men and associated lifestyle behavior.

Methods

A total of 860 women and men aged 16-25 years old of Northeast Brazil, enrolled in the POP-Brazil study, an ongoing nationwide HPV prevalence study, enrolling 7,505 participants between October 2016 and June 2017. DNA was extracted from specimens collected in Primary Care Units and HPV genotyping was performed by polymerase chain reaction amplification followed by hybridization (Linear Array Roche®). Demographic and sexual behavior were gathered by interview in the Primary care unit.

Results

The demographic characteristics of the sample reflect the general population of the region: the mean age was 20.5 (± 2.8) and the majority self-declared as brown skin color (64.4%), with household income less than US$ 500.00 per month. The overall prevalence of HPV was 56.2% (95% CI 50.0-62.4) and the prevalence of high-risk types was 38.2% (95% CI 32.1-44.2). Education level (PR=1.2; p= 0.42) and household income (p= 0.46) were not associated with high risk HPV. HPV presence was associated with smoking (PR=1.8; 0.007) and drug use (PR=1.4; 0.05) but not with alcohol consumption (PR= 1.3; p=0.15). Young people with HPV have higher number of sexual partner (PR=1.2; p< 0.01) but the age of first sexual intercourse was not associated (PR=1.04; p=0.2). Same-sex relationships did not increase the prevalence (PR=1.0; p>0.05) as well as the use of condom (PR= 1.1; p=0.54). The most prevalent types were 16 (11.2%), 58 (6.0%) and 59 (5.2%).

Conclusion
Brazilian infections by all HPV types and high-risk types in young people from Northeast region is very high. Certain lifestyle factors as smoking, drug use and number of sexual partners are associated with an increased prevalence of infection. Our data on HPV prevalence and behavior are crucial for evaluation of the effectiveness of the existing HPV vaccination recommendation in Brazil and planning of preventive strategies to reduce the burden of cervical cancer and other HPV-related tumors.
Detection of HPV- ZIKV Co-infections in Ecuadorian Women Using Two Real-Time PCR-based Methods in Cervical Cytology Samples

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Background / Objectives

Human papillomavirus (HPV) is the cause of cervical cancer. Zika virus (ZIKV) infection during pregnancy has been linked to birth defects. There is limited information about the detection of co-infections with these two pathogens.

We had the objective to detect the presence of HPV and ZIKV in cervical cytology specimens from 109 healthy women attending the Hospital Luis Vernaza (Guayaquil, Ecuador).

Methods

All samples were tested for high risk HPV genotypes (HPV-HR) using the Cobas HPV 480 and for ZIKV using a validated, laboratory-developed real-time RT-PCR (the ZCD assay). Cobas HPV has specific call-outs for HPV 16 and HPV 18 and combines detection of the remaining 12 genotypes.

Results

Patient age range was 22 to 68 years, with a median of 39.34. HPV DNA was detected in 19 women (17.43%). Eighteen (16.51%) were positive for a single marker (HPV-HR, HPV-16 or HPV-18) while one (0.9%) was infected by both HPV-16 and HPV 18. ZIKV was detected in 18/109 (16.51%) patients. Of the ZIKV positive women, the majority were under the age of 45 (13/18[JJW1] vs 5/18). We found 4 cases of HPV- ZIKV co-infection: two patients were positive for HPV-HR (ages 41 and 53) and one patient each was positive for HPV 16 (age 49) and HPV 18 (age 26). All four women were asymptomatic from the ZIKV infection.

Conclusion

These data on ZIKV detection provide additional supporting evidence for female-male transmission and demonstrated to the potential utility of screening for ZIKV in
To the best of our knowledge, this is the first report of HPV-HR-ZIKV co-infections.

References


HPV16/18 E6 ONCOPROTEIN EXPRESSION IN INFECTIONS WITH SINGLE AND MULTIPLE HPV GENOTYPES AND ASSOCIATED THE RISK OF CERVICAL DISEASE

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Background / Objectives

To characterize the likelihood of HPV16/18 to express E6 protein in single and multiple type HPV infections, and to analysis their risk of cervical disease.

Methods

Women with normal histology (n=773), CIN1 (n=62), CIN2/3 (n=130), and cervical cancer (n=466) were analyzed for presence of 14 types of high-risk HPV DNA and HPV16/18 E6 oncoprotein using BD onclarity™ and OncoE6™ assay.

Results

Of 1431 subjects, 546 (38.16%) tested positive for HPV16/18. The frequency of E6 oncoprotein expression was significantly higher in single infections than in multiple infections for both HPV16-E6 and HPV18-E6 (HPV16: 80.2% vs. 61.6%, p<0.001; HPV18: 75.7% vs. 47.6%, p=0.011). In HPV16/18 coinfection, the positivity rate was 44.4% for HPV16-E6 protein and 40.7% for HPV18-E6 protein. Only two cases showed expression of HPV16-E6 and of HPV18-E6 at the same time. The overall positivity rate of HPV16 and HPV18 oncoprotein expression in HPV16/18 coinfection subjects was 77.8%, almost the same as in single infection (HPV16: 80.2%; HPV18: 75.7%). Multiple HPV infection clusters most likely to express E6 were HPV16/52 (71.4%), followed by HPV16/51 (60.0%), and the less were HPV16/45 (14.3%). In CIN2+, E6 positivity was 86.5% for HPV16 and 89.7% for HPV18 single infection, significantly higher than 70.5% for HPV16 (P<0.001) and 56.6% for HPV18 in multiple infections (P=0.004).

Conclusion

HPV16/18 E6 is more likely to express in women with single HPV infection than in women with multiple HPV. Multiple HPV infection clusters show distinctive propensity to express E6 oncoproteins. This could relate to possible intergenotypic competition as consequence multiple infections.
Background / Objectives

Although Human Papillomavirus (HPV) is a necessary cause of cervical cancer, it is not a sufficient cause, so, others cofactors like co-infection with microbial pathogens could increase that risk. There is an association between the prevalence of high risk HPV genital infection and cervical cancer in adult women.

Methods

We studied 1067 patients mean age 37.2±9.9 (range: 17-76 years old) from multicenter hospitals for HPV detection and a sub-sample of these patients (n=172) for HPV and microorganism detection of the Gynecology/Oncology ambulatory of Santiago Hospital. The cervical samples were obtained for cytology, HPV, Ureaplasma parvum, Ureaplasma Urealyticum, Mycoplasma Genitalium and Mycoplasma Hominis detection. The method used for HPV detection and genotyping determination was Polymerase Chain Reaction followed by hybridization. The statistical methods used were Chi-square, ANOVA and binary logistic regression (SPSS v.22). Significance was attributed if P<0.05.

Results

From 1067 patients, 314 (29.4%) had HPV DNA positive among women with normal and abnormal cytology. The incidence of HPV DNA positive was highest in women aged 20-40 years old (n=196, 62.4%). We identified 33 HPV types, which HPV 16 was the most predominated (n=60 (14.6%) followed by HPV types 31 (n=39, 9.5%), 51 (n=38, 9.2%), 52 (n=36, 8.8%), 53 (n=35, 8.5%), and 66 (n=27, 6.6%). The HPV genotypes were classified in low-risk (LR) (n=39, 12.4%), high-risk (HR) (n=286, 91.1%), 2 or more HPV types (n=97, 30.9%). For molecular diagnostic tests, we found that the majority of women with HPV DNA positive presented normal cytology (n=176, 56.1%), followed by atypical squamous cells of undetermined significance
(n=100, 31.8%), 35 (11.1%) with low-grade squamous intraepithelial lesions and 3 (1.0%) with high-grade squamous intraepithelial lesions. In a sub-sample (n=172), we found that the presence of genital microorganisms was increased 3.0 times with HPV infection (OR=3.0, 95%CI [1.2-7.9], P=0.019). Furthermore, 2 or more HPV types increased this risk for 3.2 times (OR=3.2, 95%CI [1.3-8.1], P=0.015). Ureaplasma parvum was the microorganism more prevalent (71.4%); being 23.3% HPV positive and 24.1% with 2 or HR of detected HPV.

**Conclusion**

The presence of genital microorganisms was increased with HPV infection, being the presence of Ureaplasma parvum associated to HPV positive. We propose that the screen for the presence of different microorganisms could be important in prevention of severe dysplasias.
Population-based study on distribution of HPV infection and its risk factors among women in Inner Mongolia, China

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Background / Objectives

To explore the epidemiologic characteristics of human papillomavirus (HPV) infection among women from Jungar banner, Inner Mongolia, China, and analyze the related risk factors.

Methods

The cervical cancer screening program for women aged 35-64 has been conducted in Jungar banner, Inner Mongolia, China in 2016. CareHPV was applied as primary screening method followed by visual inspection with acetic acid/Lugol’s iodine (VIA/VILI) as triage method. The HPV and VIA/VILI positive women were referred to colposcopy and biopsy if necessary. Chi-square and stepwise logistic regression analysis were performed by using the SPSS21.0 statistical software.

Results

A total of 7659 women received careHPV test were included in the final analysis. Average age of the study population was (45.55±7.37) years. The overall HPV infection rate was 14.60%, and there was no difference between Han and the Mongol nationality with the infection rates of 14.54% and 15.26%, respectively (P > 0.05). The HPV infection rates in ≤CIN1 (normal or cervical intraepithelial neoplasia grade 1) and CIN2+ (cervical intraepithelial neoplasia grade 2 or higher) were 14.14% and 82.98%, (χ²=15.93, P=0.00). The HPV infection rates were different among the different age groups: 13.71% (≤44 years old), 14.26% (45-54 years old), 18.74% (>55 years old), (χ²=15.93, P=0.00). Single factor logistic regression analysis shows that the age of sexual debut (≤20 years old, with OR=1.45, 95% CI:1.23-1.72), the first childbirth age (<25 years old, with OR=1.21, 95% CI:1.04-1.39), unmarried (OR=1.76, 95% CI:1.05-2.95), education levels at high school and below (OR=1.43, 95% CI:1.18-1.74), the history of reproductive diseases (OR=1.29, 95% CI:1.11-1.49) and multiple parity (>2 times with OR=1.23, 95% CI:1.02-1.47) would increase the Han’s risk of HPV infection. However, only the first childbirth age (<25 years old) was significantly related with HPV infection in Mongol women with OR=1.60, 95%
Multi factor unconditional logistic regression revealed that the age of sexual debut, marital status, the history of reproductive diseases were significantly connected with HPV infection.

Conclusion

The overall HPV infection rate of women in Inner Mongolia was higher than that in rural areas of China. HPV positivity among women with CINII+ was significantly higher than that among women with normal cervix or CINI. Risk factors of HPV infection were different between Han and the Mongol nationality; Cervical cancer prevention propaganda should be strengthened to reduce the disease burden of cervical cancer.
Effects of vaccination on the epidemiology of HPV67 in a Belgian routine setting

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Background / Objectives

HPV vaccination programs with the HPV bivalent (HPV16/18) vaccine (Cervarix®, GlaxoSmithKline Biologicals, United Kingdom) or the recombinant HPV quadrivalent (HPV16/18/6/11) vaccine (Gardasil®, Merck & Co, Inc., USA) have been implemented in the majority of industrialized countries. According to the International Agency of Research on Cancer (IARC, World Health Organization, France) HPV67 is subdivided as a possible high-risk HPV. This study was performed to broaden the epidemiological knowledge of HPV67 in a Belgian Routine setting, and to evaluate the potential influence of vaccination status on its prevalence.

Methods

In total, 478,822 samples were evaluated with the Riatol qPCR assay. Self-reported vaccination status was known for 376,905 samples. A subset of 22,878 women reported to be vaccinated.

Results

HPV67 is found in 1.23% (95% CI: 1.20%-1.26%) of the screening population, with a significant (p<0.001, Pearson's Chi Square Test) higher prevalence in the diagnostic population 4.16% (95% CI: 4.04%-4.28%). In the screening population, multiple infections with other HPV genotype(s) occur in 51.36% (95% CI: 49.96%-52.76%). The most prevalent coinfections involve HPV39/51/16/59. Furthermore, HPV67 is associated with HPV18/39/31 in coinfections within the diagnostic population. HPV67 is significantly (p=0.021, Pearson's Chi Square Test) more prevalent in vaccinated women (3.36%; 95% CI: 3.06%-3.68%), compared to the non-vaccinated population (2.94%; 95% CI: 2.75%-3.14%). Similar results are obtained for other HPV types. In the proportion of women vaccinated with Gardasil® (0.13%; 95% CI: 0.11%-0.14%) vs Cervarix® (0.11%; 95% CI: 0.08%-0.13%) no significant differences (p=0.228, Pearson's Chi Square Test) are found.
Conclusion

The overall prevalence of HPV67 in Belgian women is 1.86%. The changing coinfection patterns between screening and diagnostic populations should be topic of further research. Specific non-HPV16/18 genotypes are observed to be significantly more prevalent in vaccinated women, irrespective of vaccine type. These data hypothesize a continuous HPV genotype shift. In addition, HPV31/33 (together with HPV16 in α9-species) and HPV45/68 (with HPV18 in α7-species) display a significant reduction in prevalence in the vaccinated population. Phylogenetic related HPV genotypes share similar capsid epitopes, potentially eliciting cross-reactive immune responses after vaccination. Our findings indicate that selected possible high-risk HPV types as HPV67 are more frequently found in vaccinated women. It is therefore warranted to perform close surveillance on the transforming potential of these types, including HPV67.
FC 20-01
ACCEPTABILITY OF SELF-SAMPLING IN NEW ZEALAND: A PILOT STUDY

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Background / Objectives

In New Zealand (NZ) there are major ethnic inequalities in cervical-cancer (cxca) screening, incidence and mortality with >50% of women diagnosed with cxca not screened and a further 11% unscreened for >3 years (the recommended screening interval). Thus, novel strategies for increasing screening participation are needed.

Internationally, offering self-sampling of cervicovaginal material has been shown to increase screening uptake but no NZ studies have yet been published. We undertook the first NZ study to examine the acceptability of self-sampling in women who self-identified as Māori (the indigenous people), Pacific Islander or Asian.

Methods

Women aged 30-69 years who had never been screened or who had been screened >6 years ago were identified through our collaborating clinic. Women were contacted by a nurse and asked to attend to: 1) examine three different self-sampling devices; 2) complete a questionnaire about the acceptability of the devices; 3) take a self-sample with ≥1 device; 4) have a cytology sample taken by a nurse (as per standard care). Samples were later tested “off-label” using the cobas HPV test (Roche).

Results

We aimed to recruit 120 women but only 56 took part. The majority (31; 56%) were of Pacific Island ethnicity, 12 (21%) were Māori, 9 (16%) were Asian, and 4 (7%) were of ‘other’ ethnicities. The median age was 39.5.

The Her Swab device was used by 51 women, 8 used the Delphi Screener, and 7 a swab-based self-sampling device (7 used >1 device).

Before trying any devices, 39 women (70%) said that they would prefer to self-sample next time they were due for screening, 11 (20%) that they would prefer a smear test, and 6 (10%) did not answer the question. After trying a device, fewer women (29; 52%) preferred to self-sample next time, slightly fewer women (7; 13%)...
preferred a smear test, 8 (14%) expressed no preference, and 12 (21%) did not answer the question.

Before using a device 29 (52%) women thought that they would experience no or very little discomfort using the device, 24 (43%) that they would experience some or a lot, and 3 (5%) declined to answer. After using a device slightly more women (31; 55%) experienced no or very little discomfort, and fewer women (16; 29%) experienced some or a lot of discomfort, but more women (9; 16%) did not answer the question.

HPV testing was unremarkable with only one sample positive for HPV “other” oncogenic types out of 32 valid tests.

Conclusion

This is the first study of self-sampling in NZ and the first study to include Māori women. Although the sample size is small, the pilot study suggests that un- and under-screened NZ women find self-sampling acceptable and all sample types are feasible for HPV testing.
EVALUATION OF HIGH RISK HPV DNA DETECTION IN SELF-COLLECTED VAGINAL SAMPLES AND URINE IN A TEST-OF-CURE SETTING

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Background / Objectives

Self-collection is considered an alternative for improving coverage rates in cervical cancer screening, while it’s use in the test-of-cure setting (ToC) is not well established. Aim: Comparing the high risk HPV DNA (HPV) status of self-sampled vaginal fluid (VF) and first-void urine (FVU) to physician-sampled cervical scrapes (LBC) collected 6 months post first life-time treatment of high grade cervical lesions during the same visit, using a PCR-based clinically validated test.

Methods

VF (Qvintip, Aprovix), FVU and cervical smears (PreservCyt, Hologic) from women with pre-treatment histology of CIN2 (N=100), CIN2/3 (N=24), CIN3 (N=250), AIS/CIN3 (N=11) and AIS (N=9) were tested with RealTime High Risk HPV (Abbott), followed by establishing correlation of results with cytology and colposcopy data. Dried FVs stored at room temperature and VFU samples, frozen within <1 h from collection, were placed into Cervi-Collect tubes (Abbott) prior to testing.

Results

Valid HPV results were obtained from all LBC samples, 99.7% VFs and 95.7% FVUs. 376 triplets with valid PCR results were available for analysis. In women with abnormal cytology (N=60), concordance between HPV results from VF/LBC (90.0%) was higher than that from FVU/LBC (78.3%); comparable HPV detection rates were found with VF/LBC pairs (55% [33] ea.), while fewer FVU samples were HPV-positive (43.3% [26]). In women with normal cytology (N=316), similarly high concordance between HPV results from VF/FVU and LBC samples was found (89.9% ea.), while a significantly higher HPV detection rate was observed with VF (21.8% [69]) compared to FVU (12.3% [39]) and LBC (13.0% [41]). In women with high grade lesions identified during colposcopy (N=29), high concordance between HPV results from matched self-and physician-collected pairs (VF/LBC 96.6%; FVU/LBC 89.7%) and comparable HPV detection rates with all three sample types (LBC 37.9%[11]; VF and FVU 41.4% [12] ea.) were found. In women with normal/low grade (NLG; N=196) and TZ3 (N=151) colposcopy, similar patterns in concordance between HPV results from VF/LBC and FVU/LBC pairs (NLG: VF/LBC 87.8%; FVU/LBC 84.7%; TZ3: VF/LBC 91.4%; FVU/LBC 92.1%) and significantly higher HPV detection rates from VF vs LBC compared to FVU vs LBC (NLG: VF 30.6% [60], FVU 18.4% [36]), LBC...
19.4% [38]); TZ3: VF 19.9% [30]), FVU 11.3% [17]), LBC 16.6% [25]) were observed.

**Conclusion**

All VF and the vast majority of FVU samples from HPV-positive women with high grade lesions at 6 months control by colposcopy were identified with RealTime High Risk HPV, suggesting that self-collected VF and FVU may be suitable for HPV-testing in the ToC-setting.
UNDERSTANDING WOMEN’S PERSPECTIVES AND INFORMATION NEEDS FOLLOWING A POSITIVE HPV SELF-SCREENING TEST RESULT

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Background / Objectives

At-home HPV self-screening with triage of high-risk HPV+ women to in-clinic follow-up may improve cervical cancer screening adherence. Understanding patient experience after a positive kit result is essential to optimize delivery and minimize negative perceptions of self-screening. We explored patient perspectives following a HPV+ self-screening result to identify information needs and emotional responses to this potential home-based screening modality.

Methods

We conducted a pragmatic randomized controlled trial in Kaiser Permanente Washington (an integrated healthcare system) to compare two programmatic approaches for increasing screening among women aged 30-64 years who were overdue (≥3.4 years since last Pap; see abstract #414 for details). Control arm included usual care (annual patient reminders and adhoc clinic outreach). Intervention arm included usual care plus an unsolicited mailed HPV self-sampling kit. We recruited 46 women who returned a kit and tested HPV+ (62% of invited; median age 55.5 years) to complete a semi-structured interview and a brief survey. Most women completed timely diagnostic evaluation (85% had a Pap and/or colposcopy, mean=15 [IQR=10-35] days between HPV+ result and first in-clinic procedure). Four coders analyzed transcripts using iterative content analysis.

Results

Seven themes emerged: 1) convenience of home test; 2) surprised by kit results because low perceived risk of HPV infection; 3) anxiety and urgency to follow up and discuss results with provider; 4) poor understanding of kit results and subsequent information-seeking through Internet, patient portal, and family/friends; 5) provider communications about results eased patient worry; 6) confusion about purpose and meaning of HPV versus Pap results; and 7) concern that HPV self-screening was inaccurate when follow-up Pap was normal. Most women strongly agreed their experience using the kit was positive; but, only 65% agreed they trusted the HPV result and 59% believed it was correct.
Conclusion

Although women liked the test’s convenience, communication about discordant home HPV and in-clinic Pap results led some to question the accuracy of self-screening. Patient-provider communication around self-screening is more complex than for reflex or co-testing, because clinician-collected Pap results are unknown at the time of the positive self-screen. Women need information about the differences between HPV and Pap tests and how findings from both are used in combination for screening and follow-up. To reassure women and keep them interested in self-screening, education is needed at three key points: when mailing the kit, releasing HPV+ results, and discussing in-clinic diagnostic findings.
Detection of cervical (pre)cancer on the basis of cervicovaginal fluid: possibilities for development of a selftest.

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Background / Objectives

Despite tremendous efforts over the last decades, current screening methods for cervical cancer still have limitations in sensitivity and/or specificity. Moreover, vaccines are not effective against all HPV types and efficiency is uncertain in case of previous infection. In the search for more specific and sensitive biomarkers, and additional challenge represents the application of these biomarkers in low- and middle income countries where the incidence of cervical cancer is highest.

The Cervico Vaginal 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 self-sampling as a good and acceptable sample collection method for subsequent DNA genotyping, cytology or immunohistochemistry, CVF may very well be suited for the development of a selftest for triage of suspected cases or screening in low- and middle income countries.

Methods

A differential proteomics study on CVF was performed using six CVF samples from healthy and six samples from precancerous women. Extracted proteins were run over a 2D-LC-MS/MS platform and quantified by spectral counting. Lists of identified CVF proteins were analyzed by Ingenuity pathway Analysis (IPA) to find out whether cervix cancer pathways were reflected in the CVF. A series of candidate biomarker proteins was further validated by ELISA or mass spectrometry (MRM).

Results

We identified alpha-actinin-4 (ACTN4) as a protein biomarker that could discriminate between the healthy and (pre)cancerous states with a sensitivity and specificity of resp. 84 and 86%. Based on the list of proteins that were differentially abundant in both types of CVF, a set of cervical cancer protein biomarkers interconnected within several cancer-related pathways was identified by Ingenuity Pathway Analysis (IPA). We quantified these biomarkers by ELISA or mass spectrometry (MRM) in CVF samples from healthy or precancerous woman in order to further increase the discriminative power in combination with ACTN4.

Conclusion
The cervical vaginal fluid may contain several biomarkers which, when used in an appropriate combination, could be used for development of an accurate cervical cancer screening test. Since collection of CVF is non-invasive, these biomarkers allow for the development of a self-diagnosis test to be used for screening, prediction or follow-up of cervix cancer.

References

PERFORMANCE EVALUATION OF A NEW SELF-SAMPLING DEVICE FOR HPV DETECTION AND GENOTYPING IN ROUTINE CERVICAL CANCER SCREENING

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Background / Objectives

The objective of this study is to evaluate the suitability of dry self-collected vaginal samples, using the lunetest® self-sampling device, for the determination of human papillomavirus (HPV) by a comparison to HPV test results obtained from standard physician-collected samples.

Methods

We included 100 patients who came for routine cervical cancer screening and 100 patients with a previous diagnosis of intraepithelial neoplasia (CIN) grade 1 or greater (CIN1+). For each patient, two samples were collected for molecular HPV testing, one obtained by the physician (cytology specimen in Thin Prep) and the other by the patient, using the lunetest® self-sampling device. Upon collection, samples were sent to the Pathology Department at the University Hospital Son Espases for analysis with PCR COBAS 4800. The presence or absence of CIN1+ was verified by colposcopy and biopsy for all women that tested positive in at least one of the samples. As a quality control measure, in cases where only one sample tested positive, both samples were retested using LINEAR ARRAY® HPV Genotyping Test (Hospital Universitario Santa Cristina). Finally, all samples were analysed for 14 high-risk HPV (hrHPV) and 2 low-risk HPV (lrHPV) types using multiplex fluorescent PCR (F-HPV typing™) at Labco Laboratories.

Results

Both sampling techniques proved to be comparable for cytological lesion detection through the detection of HPV-positive cases. The agreement between the two techniques was very good, with a Kappa index of 0.88 (CI 95%, 0.74 to 0.91) (p<0.001). One hundred and twenty-six women (63%) preferred self-sampling, mainly for its comfort (55%), which included privacy (29%) and time flexibility (27%).

Most frequent HPV types detected in both sampling methods were hrHPV-16 (27.5%), 52 (13%), 39 (9.5%), 66 (8%) and 58 (8%). Cases with a single HPV type infection were present in 47 physician-collected samples (59%) and 49 self-collected
samples (62%). Cases with multiple HPV type infection were present in 32 physician-collected samples (40.5%) and 30 self-collected samples (37.9%).

Overall result concordance between the two sampling procedures was 90%, 99% for HPV-16, 99% for HPV-18 and 99.1% for the remaining hrHPV types.

Conclusion

Dry self-sampling is a valuable alternative to obtain samples for primary cervical cancer screening due to its reliability, comfort and low cost. As we know, most cervical cancer cases occur in women that do not attend screening. Self-sampling can not only cut costs of screening but also motivate more women to participate in cervical cancer prevention programs.

References


URINE HUMAN PAPILLOMAVIRUS HOME SELF-SAMPLING, A PROMISING STRATEGY FOR ENHANCEMENT OF UTERINE CERVICAL CANCER SCREENING IN A LARGE RURAL FRENCH COHORT WITH A 5-YEAR CLINICAL FOLLOW-UP (THE PAPU29 STUDY)


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Background / Objectives

To increase patient’s compliance with cervical cancer (CC) screenings, urine self-sampling HPV-DNA detection could be an alternative to clinician sampling (1). Home self-sampling can reduce screening difficulties, CC incidence and mortality. The aim of this French prospective PapU29 study was to evaluate self-collected urine HPV-DNA screening in 25-65 yo rural women non-attenders to organized cytological-based (OC)-CC screenings.

Methods

From 2008 to 2010, 15471 women were invited to cytology screening. All the responding women were assigned to “cytological first-line” (CFL) group. A urine HPV-DNA kit was sent to those non-responders at home (“HPV first-line” (HFL) group). Urine sample was send by mail to the virology labo from Brest university hospital for HPV DNA quantification by real-time PCR (2,3) and genotyping (InnoLiPA). Women with positive test were assigned for “cytological second-line” within 3 months, those with negative test were recommended for a cytology within 3 years. Participation rate was compared between CFL and combined CFL-HFL. The HFL group was followed-up for 5 years (2011-2016). This study was supported by the French "Ligue contre le Cancer". All women gave their signed consent.

Results

Participation rate (15260 eligible women, mean age: 46.6 yo (11.5)) was significantly increased between CFL and combined CFL-HFL (3.73% vs. 31.9%, p < 0.001) (> 50 yo women: 4.67% vs. 33.3%, p<0.0001). After urine HPV screening, abnormal
cytology detection rate was increased (4.11% vs. 6.31%, p=0.078). Screening rate of precancerous lesions CIN2+ was increased (0.41% vs. 1.42%, p=0.11) (35-50 yo women: 2.55%, p=0.038). First case of HPV18 adenocarcinoma was identified in HFL. The most high-risk genotypes (HPV16, 18) were detected in positive HPVs with CIN2+ (p=0.019). The 5-year follow-up showed no CIN2+ in negative HPVs and only one in positive HPVs with normal cytology at the baseline.

**Conclusion**

The feasibility and reliability of first-void urine HPV home self-sampling was showed in rural women non-attenders in OC-CC screenings. The high rate of CIN2+ detection demonstrated the clinical benefit of using urine HPV in women at high risk of CC. Given today's rise in HPV vaccination, we propose urine self-collect HPV as a primary CC screening strategy and a 5-year urine HPV screening interval. We recently found comparable results with vaginal self-sampling and a 3-fold higher acceptance for urine sampling (on-going analysis). Further large-scale randomized studies on difficult to reach women are warranted to validate our results with urine HPV screening.

**References**


URINARY HPV DNA TESTING AS A TOOL FOR CERVICAL CANCER SCREENING IN FRANCE

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Background / Objectives

Previous studies have shown that self-sampling for human papillomavirus (HPV) testing increases rates of compliance. With this purpose in mind and in collaboration with Cap Santé 49, we performed the CapU 1-2 studies to evaluate the acceptance of an urinary HPV test. Letters proposing an at-home urine self-sampling were sent to 5,000 women who not had a Pap smear over the past 3 years. The participating patients had to send their urine samples to the Angers Hospital Virology Laboratory. High-risk HPV (HR-HPV) were detected in 29 women using real time PCR. In follow-up, 28 women with positive urinary HPV results had a Pap smear or colposcopy done. The cytological results showed 9 abnormal Pap smears, among which histology studies confirmed 3 cases of cervical intraepithelial neoplasia grade III lesions (Ducancelle et al, 2015).

Since September 2016, a 3rd study, CapU3, aims to invite 13,000 women aged 35 to 65 who had not had a Pap smear over the past 7 years. 500-700 letters proposing an at-home urinary HPV testing are sent monthly. With the letter, the women receive an information note, a letter of consent, a sterile container, a procedure protocol, a bubble envelope and a prepaid return envelope. Women accepting to participate send their first-stream urine samples by mail to the Angers University Hospital Virology Laboratory using the bubble envelope and the prepaid envelope in accordance with a three-rule secure packaging protocol as recommended in France. The end of the study is scheduled for November 2018.

Methods

HR-HPV detection is performed using a real-time PCR (Anyplex II HPV28 Detection) that detects 28 genotypes. Patients with HPV positive results are encouraged to perform a cervical smear as soon as possible to detect the presence of cervical lesions. For HPV-negative women, a Pap smear within 1 year is recommended for those women who do not have regular gynecological follow-up.

Results

The preliminary response rate is 15.9% (635/4,000). After exclusion (for hysterectomy or refusal), the participation rate is 13.3% with 530 samples received. Among them, 492 specimens were already tested in which 58 were positive for at least 1 HR-HPV (11.8%). Among the cervical smears performed in positive patients, 1 high-grade cytological lesion has already been detected.
Conclusion

Because home HPV urinary testing are non-invasive and do not require medical attention, this method may be an alternative for women who are reluctant to use Pap smear. In the other hand, we show that Anyplex II HPV28 Detection is a valuable assay for urinary HPV testing as described.

References

IS HPV E6 ONCOPROTEIN DETECTABLE IN URINE AMONG WOMEN WITH INVASIVE CERVICAL CANCER?

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Background / Objectives

Cervical cancer (CxCa) is a major public health problem, especially in low- and middle-income countries (LMICs), where women have little access to CxCa screening; consequently 80% of CxCa related mortality occurs in LMICs. The development of screening methods that need less infrastructure thus represents an urgent medical need.

Objectives: To evaluate the feasibility of detecting HPV16 and/or HPV18 E6 oncoprotein in urine samples from women with invasive cervical cancer (ICC).

Methods

Between January 2017 and April 2017, sixteen women with previously untreated macroscopic ICC (FIGO stage IB or greater) with ages ranging from 25 to 64 years were recruited at the Barretos Cancer Hospital – Pio XII Foundation. At study enrollment, self-collected vaginal samples, physician-collected cervical samples and urine samples were obtained prior to colposcopy. Urine-based protocols to measure HPV-DNA via Cobas® HPV Platform (Roche, CA, USA) and E6 oncoprotein levels using the OncoE6™ Cervical Test (“E6 test”; Arbor Vita Corp., CA, USA) were developed and applied.

Results

The mean age of participants was 43.2 years. All vaginal self-collected samples tested positive for high-risk (HR) HPV-DNA: 11 for HPV16, 2 for HPV18, 5 for other HR-HPV types; 2 had multiple infections. Fifteen (93.8%) physician-collected cervical specimens and urine samples tested positive for HR HPV-DNA, with the same genotyping results observed in correspondent self-collected vaginal samples. Among HPV16 or HPV18 positives, corresponding E6 oncoprotein was detected in 10 of 13
vaginal self-collection samples, in 12 of 13 physician collected samples, and in 7 of 13 urine samples. HPV E6 oncoprotein types showed 100% correlation to HPV genotyping outcome.

Conclusion

Our results suggest that vaginal self-collection may be compatible with the E6 Test. Surprisingly, E6 oncoprotein was also detected in urine of ~54% of women with ICC, suggesting that a urine based E6 Test may be possible upon further protocol development. Vaginal and urine based detection of E6 oncoprotein could enhance CxCa screening coverage in LMICs.
Background / Objectives

Indigenous Māori women experience substantially higher rates of cervical cancer than New Zealand European women do. The National Cervical Screening Programme (NCSP) consists of a three yearly Pap smear for women aged 20-70. However, this is an invasive and sometimes time consuming and/or costly procedure, and screening rates are significantly lower for Māori. HPV self-sampling presents an opportunity to overcome these barriers. This project explores the acceptability of HPV self-sampling for under-screened Māori women (aged >25 years, with no screen in >4 years). This research is funded by the New Zealand Ministry of Health and will inform the new NCSP in 2018.

Methods

This Kaupapa Māori (by Māori, with Māori, for Māori) mixed methods project explores the attitudes and beliefs towards HPV self-sampling of an under-screened Indigenous population using a distributed research model (DRM). This involved community based researchers (CBRs) and community members collecting the data. CBRs ran 19 focus groups/interviews with 94 under-screened Māori women in four regions. Preliminary focus group/interview data was analysed thematically and organised into potential barriers or facilitators to accepting this new technology. The analysis guided the development of a short survey that focus group participants disseminated to peers. Percentages were calculated from the survey data.

Results

Potential barriers to self-sampling were: concerns about accuracy/safety, concerns about the sample getting lost/contaminated, stigma of HPV being an STI, and concerns about the longer re-testing time (5 years if negative). Facilitators were: privacy/ease, competent practitioners, appropriate information, whole family approach. Survey data (to-date) shows: 90% of participants are enrolled with a Primary Health Organisation, 75% are likely/very likely to self-sample, and 90% are likely/very likely to seek further diagnosis if their HPV test is positive. The DRM has
been successful in engaging 309 eligible (considered hard to reach) survey participants.

Conclusion

The majority of under-screened Māori women who took part in this study are engaged in the health system, but they do not screen. This is a system failure. Both the focus group and survey data emphasise the acceptability of HPV self-sampling for under-screened Māori women, if accompanied by appropriate support. Almost all participants indicated they would seek further diagnosis or treatment if required. This suggests that with well introduced self-sampling, many currently under-screened Māori women would be screened (and treated if necessary). HPV self-sampling has the potential to save lives.
SELF CERVICAL COLLECTION FOR HPV, HHV-2 AND HIV-1 DETECTION IN WOMEN FROM LOWER AMAZON


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Background / Objectives

Self-cervical collection and epidemiological studies on anogenital infections by human papillomavirus (HPV), human herpes virus 2 (HHV-2) and human immunodeficiency virus 1 (HIV-1) in women from outlying regions to urban centers, such as the interior of the State of Pará, are limited. Objective: To estimate the prevalence of anogenital HPV and HHV-2 infections in association with, or not, to HIV infection and to verify the uptake of self cervical collection.

Methods

Recruitment of a cross-section of women who voluntarily sought assistance in eight different health services provided by the municipalities in Tapajos/Santarem on the lower Amazon in Pará, Brazil. The study utilized swabs from the cervix and anus along with peripheral blood obtained by trained technicians that were compared to samples from self-administered cervical collections. All specimens were preserved in Thin Prep and collected between August 2015 and August 2016. PCR products amplified by MY09/11 and GP5/6+ primers were sequenced to genotype HPV DNA. Real time PCR was used to detect HHV-2 nucleic acid.

Results

A total of 476 specimens from 112 women were analyzed. A high acceptance to the self-administered cervical sampling protocol was shown by the study population (84%, 94/112), which identified HPV DNA in 37 samples (39.4%). The most prevalent HPV type was HPV16. The prevalence of HHV-2 was 8.9%. The agreement rate between the clinical samples and the self cervical sampling was 65% (26/40) for HPV and 50% (4/8) for HHV-2. No HIV-1 infected women were detected.

Conclusion


A high prevalence of oncogenic HPV type with no dysplastic lesion was found in cervical and anal samples. The self-cervical collection had a high acceptability, especially among vulnerable women, that strongly suggests that this approach could be an important tool to increase access to diagnosis. We encourage public healthcare officials to include self-administered samples in future screening programs for cervical cancer and the identification of circulating STDs in women that live in low-resource and rural settings, such as the Amazon region of Brazil.

References

UTILITY AND FACTOR EVALUATION OF HPV DETECTION BASED ON URINE SAMPLES IN GENERAL CHINESE WOMEN

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Background / Objectives

Cervical cancer screening is mainly based on cervical exfoliated cell samples at present. To reach out women without access to routine screening, self-collection could be one promising alternative sampling choice. Urine sample collection seems to be convenient and lower recourse demand. The utility and impact factors of HPV testing from urine samples were evaluated in subset population in a pilot HPV primary screening study in China.

Methods

34-64y women from Yangcheng and Xiangyuan in Shanxi Province consented to participate. Women self-collected at least 40ml urine sample and mixed with 10ml EDTA preservative before stored at 4°C. Another clinician-collected cervical sample was placed in ThinPrep™ Solution (Hologic, Inc., Marlborough, MA) or DCM solution (Qiagen, Gaithersburg, MD). Urine samples were tested with the Trovagene high-risk HPV assay (Trovagene, Inc., San Diego, CA) and cervical samples were tested with the cobas® HPV Test (Roche Molecular Systems, Pleasanton, CA) or the careHPV™ test (Qiagen, Gaithersburg, MD). At the time of urine collected, information were also obtained through face-to-face interview, including urinary frequency, time interval from the latest urination, intraday water intake and time interval from the latest drinking at home, water intake on site and urine collection times. Women were triaged, referred to colposcopy per the pilot study protocol and biopsies were obtained if clinically indicated.

Results

In total, 2038 women were enrolled. 1953 women were qualified as evaluable subjects and 85 women were excluded due to invalid urine testing results. The median age was 48 years old. And the HPV positive rate in cervical samples was higher than that in urine samples (18.5% vs. 16.7%, P=0.049). And the agreement rate was 84.7%. It was also indicated that intraday water intake (OR: 1.668, 95%CI: 1.196-2.325) could increase HPV positive rate. Whereas urinary frequency (OR: 0.699, 95%CI: 0.522-0.935) and drink water on site (OR: 0.616, 95%CI: 0.386-0.981). No significant differences were observed among other factors.

Conclusion
HPV positive rate in urine samples appears to be comparable to that in cervical samples. Intraday water intake, urinary frequency and water intake on site could affect HPV results in urine samples. Thus, it can be inferred that HPV detection based on urine still need to be optimized before applied in cervical cancer screening.
LONGITUDINAL STUDY OF HPV DETECTION IN PLASMA OF WOMEN WITH A RECENT HISTORY OF CERVICAL DYSPLASIA

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Background / Objectives

The presence of viral DNA offers a specific tumor marker for viral associated cancers. Some studies have reported the presence of HPV DNA in sera and plasma of women with cervical cancer, indicating the possible entry of cancer cells in circulating blood. On the contrary, only few studies have investigated the presence of HPV DNA in the bloodstream of patients with low grade or precancerous cervical lesions. The aim of this pilot study was to investigate the presence of high-risk HPV (hrHPV) in cervical and plasma samples of women with a recent history of low-grade cervical dysplasia (ASCUS or L-SIL) and to evaluate its persistence in bloodstream after 6 months.

Methods

Blood and cervical samples were obtained from 28 women referred to San Gerardo Hospital, Monza, Italy with a recent history of ASCUS or L-SIL. Routine cervical cytology (Pap test) and hrHPV detection was performed on cervical and plasma samples. HPV detection in plasma and cervical samples was also evaluated at a follow-up visit after 6 months. Nucleic acid extraction was performed using automated NucliSENS easyMAG system (bioMérieux). A full high-risk HPV genotyping assay (Papilloplex, GeneFirst) was performed on cervical samples. 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; all assays were validated through the participation to the WHO LabNet HPV Proficiency Study.

Results

At baseline, positivity for one or more of the tested hrHPV types was demonstrated in 75% (21/28) of cervical samples with the most prevalent genotypes identified being HPV 16 (37.5%), HPV 51 (25%) and HPV 31 (10.7%). Overall, 8 women (28.6%) were found to be hrHPV positive in plasma at a least one time point. The same hrHPV type was detected in plasma and cervical samples at the same time point in 31.2% (5/16) of tested samples. Five women resulted positive for the same hrHPV type at both baseline and follow up; of these 3 showed HPV DNA persistence in plasma in spite of viral clearance from the cervix.

Conclusion
These preliminary results confirm that HPV DNA can be detected in peripheral blood samples of women with a recent history of cervical dysplasia. This pilot study has also demonstrated that HPV DNA can persist in bloodstream for up to 6 months and that viral DNA can be detected in plasma even after clearance of cervical infection. 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.
Suggesting ideal strategy of cervical cancer screening in Japan based on Fukui cervical cancer screening study

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Background / Objectives

The induction of HPV testing in cervical cancer screening has spread throughout the world for the detection of cervical cancer precursors.1-3 But an ideal strategy unified in the world is not determined yet. Therefore, this Fukui Cervical Cancer Screening (FCCS) study has two important objectives. One is to confirm the performance of cytology testing, human papillomavirus (HPV) testing, and co-testing with cytology and HPV testing in Japan. The other is to determine whether the different approach by HPV16 type, HPV18 type, and 12 other high-risk HPV (hrHPV) types is a beneficial method for the Japanese cancer screening population.

Methods

The study enrolled 7,584 women aged ≥25 years who were undergoing routine screening. All women underwent cytology and HPV testing. Women with abnormal cytology regardless of the HPV status, those with positive hrHPV results regardless of cytology results, and those randomly selected from among women with normal cytology and negative hrHPV results were referred for colposcopy. This study had four features: a) all the samples were liquid-based cytology samples b) the cobas 4800 HPV test, which can detect HPV16, HPV18, and 12 other hrHPV types separately, was used c) bias in the cytological diagnosis was very small because the cytology was evaluated in a single institution d) a central pathology review panel determined the histological diagnosis.

Results

The prevalence of hrHPV types, HPV16 type and HPV18 type was 6.8%, 1.2%, and 0.5%, respectively. Estimated sensitivities for cervical intraepithelial neoplasia grade 2 or worse for abnormal cytology, positive hrHPV, abnormal cytology or positive hrHPV either, and abnormal cytology or positive HPV16 either were 71%, 92%, 100%, 86%, respectively. Estimated specificities for abnormal cytology, positive hrHPV, abnormal cytology or positive hrHPV either, and abnormal cytology or positive HPV16 either were 33%, 21%, 21% and 33%, respectively. Abnormal cytology or positive HPV16 type either is higher sensitivity and higher specificity than only abnormal cytology.

Conclusion
The FCCS study is first clinical trial to determine the performance of Japanese cervical cancer screening. This study demonstrated that a cervical cancer screening strategy to perform colposcopy and biopsy for women either with abnormal cytology or with HPV16 genotype might have a good balance between benefit and potential harm in Japan.

References


EMERGING TECHNOLOGIES IN CERVICAL CANCER SCREENING, THE ETICCS INITIATIVE

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Background / Objectives

Cervical cancer is 18 times more common in the poorest as compared to the most advanced countries affecting women in the prime of their lives and weakening the development of their communities. While the knowledge and the technologies to effectively prevent cervical cancer are available as shown in affluent societies, the dramatic disparity largely results from the lack of effective screening programs for fragile and unreliable health systems often found in low and middle income countries.

Methods

ETiCCS (Emerging Technologies in Cervical Cancer Screening) is a non-profit initiative within the Department of Applied Tumor Biology, Institute of Pathology, Heidelberg University, which aims to advance and promote organized cervical cancer screening in underserved communities with a high cancer burden. Cervical cancer screening programs will only be successful if they are broadly accessible, acceptable and using effective screening tools. Leveraging emerging technologies and novel insight into the cervical cancer pathogenesis it is possible to develop organized screening programs also for remote settings. Various new tools are available to create innovative approaches including molecular screening methods, self-sampling devices, digital imaging devices and versatile electronic systems for program monitoring and evaluation.

Results

Our current scope of activities -in partnership with the WACA HPV initiative of the Global Health Institute, University of Antwerp- include (i) capacity building in 8 sub-Saharan countries, (ii) a long-standing collaboration with SAP™ to develop new ancillary tools to combine mobile and cloud-based information technology and (iii) studying innovative, scalable screening approaches based on primary HPV testing using home-based self-sampling and (iv) studying triage options including biomarker and cervicography.

Conclusion
The ETiCCS initiative is dedicated to curb the high cervical cancer burden in underserved and disadvantaged communities through organized, scalable cervical cancer screening by forging alliances with national and international stakeholders and key industries and by developing knowledge, capacity, and advocacy. Website: www.eticcs.org
AN ELECTRONIC DATA SYSTEM FOR AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM IN A RURAL SETTING IN ETHIOPIA

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Background / Objectives

Primary HPV testing using home-based self-collection of the HPV sample is a promising screening approach for settings with a high burden of cervical cancer. The efficacy of such an approach depends on a high participation rate and a seamless follow-up of at risk women. As part of the ETiCCS (Emerging Technologies in cervical Cancer Treatment) initiative (www.eticcs.org) we developed and implemented a digital data management system that allows close monitoring of the screening process from sample collection at home to laboratory testing and clinical management for a rural community in the Gondar region of Ethiopia.

Methods

A digital prototype was developed with unique user interfaces for the community health worker, clinic nurse, lab technician, gynecologist and pathologist.

Sampling information captured by the community health worker during a home visit are stored on tablets and regularly synchronized with a clinic-based tablet at the time of sample delivery. The clinic-based tablet serves as a multidirectional hub for clinic, lab and referral information through synchronization to a central server to which the laboratory and gynecologist are also connected via browser. Server data can be accessed and corrected with audit trail by authorized person. Multilevel security includes password protection for users and encryption of sensitive private data.

Results

1000 women are being recruited in 2 rural communities in the Dabat district, Gondar, Ethiopia. Eligible women are visited in their home and invited to provide a self-sample for HPV testing. HPV-DNA positive women are invited to the local clinic for p16INK4a /Ki-67 testing. Samples are assayed in a central lab and women with abnormal findings are referred to the gynecologist for clinical management.

Conclusion
The built prototype greatly improves access and linkage to cervical cancer screening programs using home-based HPV self-sampling. The prototype is ideal for scale-up of an organized screening approach in rural settings.
The impact of migration on cervical screening behaviour

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Background / Objectives

The incidence of cervical cancer in Eastern Europe (EE) is significantly higher than Western Europe (WE) despite the introduction of screening and vaccination programs in many EE counties. The migration of women from EE has been hypothesised to contribute the rising incidence of cervical cancer in WE. The aim of this study was to explore the effect of migration to the UK on the cervical screening behaviours of EE-born women.

Methods

A mixed methods study using quantitative surveys and in-depth semi-structured qualitative interviews was conducted in the UK and Latvia. Women were recruited from three groups, migrant EE-born women (nEE), native English-born Caucasian women (nEN) and native Latvian-born women (nLV). Data were analysed using SPSS software and thematic analysis.

Results

489 surveys were completed and 66 interviews were conducted. Knowledge of the purpose of cervical cancer screening was lower in the nEE and nLV groups compared to the nEN. The nEE and nLV women believed that a cervical smear test was performed as part of a routine gynaecological examination. The natural history of cervical cancer and its association with HPV infection was poorly understood resulting in some women from nEE and nLV groups requesting more frequent smears. There was general distrust of the healthcare system in the country of migration and consequently there was a delay in engaging with screening services. nEE women either continued to have screening in their country of birth, have screening in England and additional smears in their country of birth and others did not participate in any form of screening. The screening behaviours and knowledge of the nEE and nLV group were similar, suggesting that there is little change following migration. However the length of stay in the country of migration may contribute to how much the nEE women adapt their screening behaviours.

Conclusion

The role of cervical cytology as part of a structured screening programme is poorly understood. The screening behaviours of many nEE women appears to be governed by their pre-existing knowledge of cervical cancer and screening prior to migration.
Targeted education both prior to and after migration may help to increase screening coverage.
EVIDENCE FOR CLINICAL APPLICATION OF EXTENDED HPV GENOTYPING IN CERVICAL CANCER SCREENING PARADIGMS: A SYSTEMATIC REVIEW

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Background / Objectives

Screening guideline originators (WHO, ASCO, country-specific) have not yet included an analysis of the body of science published during the last decade about the clinical value of extended HPV genotyping (xGT) in cervical cancer screening and triage, needed to support guideline panels’ recommendations. This targeted systematic review addresses key questions (KQ) that pertain to the effectiveness of including xGT results with screening for reducing cervical cancer mortality and incidence. KQ1 compares xGT versus partial genotype reporting/masking of a pool of other genotypes. KQ2 evaluates xGT as triage of primary HPV positive results, of ASCUS cytology, and as triage of cotesting.

Methods

We searched the Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews, PubMed, and the Health Technology Assessment database from 2000 through 2017 for relevant controlled trials and observational studies. We supplemented by hand-searching of retrieved article reference lists. Eligible studies included prospective studies of women and retrospective studies of residual specimens from women that were screened or tested using human papillomavirus DNA tests. The reference standard was cervical intraepithelial neoplasia 2 (CIN2) or CIN3 or CIN2+ or CIN3+ or invasive cervical cancer (squamous and/or adenocarcinoma). The timeframe was baseline, or 1-year, or 3-year, or 5-year, or greater than 5-year. Relevance screening, data extraction, risk of bias analyses, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the QUADAS evidence-based quality assessment tool of diagnostic accuracy studies, supplemented by the STARD checklist and GRADE methodology.

Conclusion

The available evidence supports the conclusion that reporting xGT results supports discrimination of both current and future CIN2+ risks. Guideline panels must decide whether to separate reporting by individual genotypes or to group genotypes with similar risks into risk tiers. Based on large studies, xGT appears very promising as
triage in all screening paradigms to discriminate risk and support risk-based clinical action steps by the principle of equal management for equal risk. Models for different screening paradigms, including routine screening interval, enhanced screening interval, referral to colposcopy, and treatment are described. The information in this report is intended to help guideline panels, policymakers, clinicians, and women make informed decisions about the selection of health care screening and diagnostic services, is intended as a reference, and not as a substitute for clinical judgment.
HIGHER RATE OF HISTOLOGICALLY CONFIRMED CIN 2+ WITH INCREASING USE OF HPV, LIQUID BASED CYTOLOGY AND p16/Ki-67 IN ROUTINE

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Background / Objectives

Annual conventional cytology (cc) is still the standard in the German cervical cancer prevention program. For the external quality control by the association of health insurance physicians (KV) a yearly report of the distribution of the histological results relating to the PAP groups is required. The sensitivity of a single cc for the detection of cervical intraepithelial neoplasia of grade 2 or higher (CIN 2+) is low. However, within the last decade HPV testing, liquid based cytology and new biomarkers (e.g. p16/Ki-67) have made it into routine use mainly in the triage of borderline and low grade cytological findings.

Methods

Cytomol is a commercial lab specialized in cervical cancer prevention. Here since the year 2000 HPV testing has been used at a progressive rate in triage and to a much lesser extent as a self-paid service in addition to cc. Since 2007 p16 testing, from 2010 on coupled with Ki-67 staining (all in liquid based cytology), is used in the triage of borderline and abnormal cytology as well as in cases with HPV positivity and normal cytology. Here we report the rate of histologically confirmed CIN 2+ cases correlated with the preceding cytological findings. Cytological diagnoses originally reported in the Munich Nomenclature II (MN; with the use of the unofficial 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). All the yearly Cytomol reports from 2007 till 2015 to the KV are analyzed.

Results

The finding rate for CIN 2+ lesions per year [Pap > ASC-US] in the screened population increased from 2007 until 2015 from 0,059% to 0,44%. The percentage of histological reports related to the different cytological groups remained almost the same over the years. The most significant increase was observed from 2007 until 2011. In parallel, a strong rise in cases of p16/ Ki-67 [2007: 292 p16, 2015: 6130 p16/Ki-67] diagnostics performed in the lab occurred. In parallel a systematic mode of review in cases with a history of abnormal cytological, histological, HPV and chlamydia findings and of actual borderline or abnormal cytology specimens had been established.

Conclusion
The introduction of new diagnostic techniques and/or improved cytological approaches in a routine lab for cytology seems to lead to a significantly increased sensitivity and specificity for CIN 2+. 
The Study of Folate Receptor-Mediated Staining Solution (FRD™) Used for Cervical Cancer Screening

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Background / Objectives

To evaluate the significance of the Folate Receptor-Mediated Staining Solution (FRD™) when used for cervical cancer detection, by comparing it with the results of the thin-prep cytology test and high risk HPV test and using pathological results as the gold standard.

Methods

The FRD™ is a special staining method for rapid visualization of CIN2+. Results are determined by the color changes of the stain. The FRD™ was applied on the cervix and the cervical canal separately, and the color change was observed. Brown or green indicates a negative result, and blue, bluish-black, or black indicates a positive result. The women included in this study were returning for evaluation by colposcopy based on an abnormal cervical cytology result, a positive HPV result, or showed symptoms of increased leucorrhea discharge or postcoital bleeding. The FRD™ test was performed before colposcopy. A biopsy, and histopathological examination were conducted as needed. Patients with a positive FRD™ result of the cervical canal, colposcopy assessment type is II-III, or the result of cytology was AGC, were required to complete an ECC as well.

Results

1,504 women with histological findings were included in the study. CIN2+ was found in 561 patients (37.3%) including 50 patients with cervical invasive cancer (3.3%). CIN1 and negative cases accounted for 29.3% and 33.4%, respectively. Pap results included NILM in 394 women (26.2%), ASC-US in 476 women (31.6%), LSIL in 334 women (22.2%), ASC-H in 96 women (6.4%), AGC in 10 women (0.7%), and HSIL in 194 women (12.9%). The HPV positive rate was 89.0% (1338/1504). A positive FRD™ test was determined in 54.1% of the women (813/1504). The sensitivity to detect CIN2+ lesions for TCT, HPV, and FRD™ was 80.4%, 95.5%, and 77.7%, respectively. The specificity was 30.1%, 15.0%, and 60.0% respectively.

Conclusion

From this study we found that the results of the FRD™ and the cytology examination are similar when detecting CIN 2+. The FRD™ can indicate the tumor tendency of epithelial tissue in direct proportional to the organization of the morphological changes. Furthermore, the more serious the nature of lesion and the higher the level,
then the more accurate the results of the FRD™ were. Also, this study shows that the FRD™ results can accurately reveal the level of the epithelial tissue lesions.

Finally, in this study, we compared the sensitivity and specificity of the FRD™ to the cytology examination, and found that the FRD™ can meet the demands of clinical requirements needed for the detection of abnormal cervical lesions (CIN 2+). Furthermore, the FRD™ is an easy and very inexpensive method that can be used in less-developed countries.
FC 21-08
CERVICAL CANCER SCREENING PARTICIPATION IN BELGIUM 2006-2012

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Background / Objectives

In Belgium, in 2012, cervical cancer (CC) screening was still opportunistic. We estimated the proportion of women aged 25 to 64 who participated in CC screening in 2006-2012 using individual health insurance data.

Methods

Data were provided by the Intermutualistic Agency (IMA), that compiled a database including all reimbursement records for screening Pap smears performed in Belgium between 2006 and 2012. Coverage was defined as the proportion of women from the target population who had a Pap smear taken within the last 3 years. Overuse was defined as the proportion of Pap smears taken that does not contribute to the coverage: (number of smears taken in 3 years / number of women screened in that period – 1)*100.

Until July 2009, reimbursement was not conditioned neither by age nor by screening interval, as recommended in European or Belgian guidelines (1 Pap/3 years in age group 25-64). Since July 2009, reimbursement was restricted to one Pap smear per two years.

Results

From 2006 to 2012, the coverage dropped from 58.7% to 53.7%. Overuse decreased from 80.5% in 2006, to 18.3%, in 2012.

In the age group 25-44, the coverage varied between 59.1% and 63.6%. The coverage dropped progressively by age (to 35.9% at age 60-64) and was lower among socially vulnerable groups benefiting from increased reimbursement: 41.6% versus 55.7% among other women. 91% of Pap smears were taken by gynaecologists and 9% by general practitioners.

Conclusion

CC screening coverage in Belgium is moderate and tended to decrease over recent years. Restriction of reimbursement diminished over-use. Organised measures are needed to optimise coverage over all levels of the target population.
FC 21-09
Cervical cancer screening in Flanders

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Background / Objectives

In June 2013, the Flemish cervical cancer screening program started. Before 2013, screening was essentially opportunistic. A efficient call recall system was set up, only inviting women who are not adequately screened. The screening program motivates eligible women 25-64 y to be screened every three years, by means of a PAP smear.

Methods

The Belgian Cancer registry collects the test results of all cervical samples in a central cyto-histopathological registry and retrieves from the IMA/AIM reimbursement data of clinical acts which are relevant for the detection, monitoring and treatment of cervical cancer. Hence, the Cancer registry compiles an exclusion list that consists of all women for which a screening examination is not required for the next invitation round. Hence, the Centre of Cancer screening will exclusively invite women who are eligible for screening.

Results

The program faces several challenges:

Coverage remains stable around 63%. 37% women are never or rarely screened. Research into the socio-demographic characteristics of non-responders demonstrated an significant social gradient. Furthermore, Eastern Europe migrant women are under screened. Targeting disadvantaged and migrant women will be necessary to reduce inequity. Currently, qualitative research among women from low socio-economic status, is conducted to explore barriers to screening. Post-menopausal women do not seem to be aware of their risk of cervical risk. After 55y coverage drops to 50%.

The relationship between disability status and screening is currently under investigation. 25% of women with screen-detected abnormalities do not receive adequate follow up. A fail safe system will be set up. Since 2010, the Flemish government has offered free HPV vaccinations to all girls in the first year of secondary education. About 82% of girls aged 13 are being vaccinated. In 2022, cohorts of young vaccinated women will enter the target population for screenin

Conclusion

- Targetting never of rarely screened women remains a priority.
- Changes in screening strategy (screening test, screening interval) may be necessary once women who were offered HPV vaccination, will reach the age of 25y.
Cervical Cancer Screening in Iran: Developing a New Method

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Background / Objectives

Cervical cancer incidence and mortality are low in Iran but the prevalence of HPV infection is increasing. On the other hand, there is no national population based program for screening of cervical cancer in Iran and a large number of women did Pap smear test based on the gynecologist prescription even annually and with different interval and inadequate accuracy. Ministry of Health of Iran planned a National cervical cancer early detection and screening Program from 2017.

Methods

Women aged 30-69 called to health care service centers in the first phase in four cities and were evaluated using cytology and HPV DNA test assays in cervical samples. In the next phase and based on new evidence and expert panel recommendation the age range of program decreased to 30-49. All samples were sent to a national lab we prepared for this purpose in Tehran. HPV DNA test was accomplished recruiting automated devices for purification, master mix preparation and electrophoresis steps. The viral DNA was amplified by conventional polymerase chain reaction using the PGMY09/11 set of primers. Experimental validation was performed for HPV DNA test according to the WHO HPV laboratory guidelines. Standard HPV plasmid of different genotypes and human cervical cancer cell lines (Hela and Ca-ski) was used to determine the analytical sensitivity of the assay. Real time PCR was used to assess the type-specific prevalence of high risk (HR)-HPV genotypes; HPV-16 and HPV-18 as predominant associated agents of cervical cancer.

Results

Among 20,000 cases tested from April 2017 in four cities, around 1400 cases were detected positive for HPV infection of any genotype using PCR-based HPV test, which is account for 7% of HPV infection prevalence. At the time of preparation of this manuscript the result of genotyping is not ready but We scheduled to do assay for 1000 cases daily so the presented results will update for the presentation we can present the result of more samples from more cities and genotyping in October.

Conclusion
We have just launched a national cervical cancer screening program with HPV testing and Cytology in Iran as the vast member in Middle East. We plan to up scale the program whole country step by step and with more automated method that help us prevent cervical cancer occurrence and decrease its mortality.
ASSOCIATION BETWEEN INTEGRATION OF HIGH-RISK HPV GENOMES DETECTED BY MOLECULAR COMBING AND THE SEVERITY AND/OR CLINICAL OUTCOME OF CERVICAL LESIONS

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Background / Objectives

High-risk human papillomavirus (HR-HPV) are causally associated with cervical cancer. 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 integration of HPV 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 EXPL-HPV-002 study is to evaluate the integration of 14 HR-HPV (16/18/31/33/35/39/45/51/52/56/58/59/66/68) by Molecular Combing as a biomarker of the severity and/or of the progression of cervical lesions.

Methods

The EXPL-HPV-002 prospective multicentric study will enroll about 600 women aged 25-65 in 2 clinical sites in the Czech Republic, referred to colposcopy after an abnormal Pap smear.

The study will be divided into two phases: (1) a transversal phase which will study the association between HPV integration status and colposcopy results and histological grades; (2) a longitudinal phase which is expected to last 36 months. This 2nd phase will study the association between HPV integration status and the progression of the lesion / infection.

HPV genotyping and Molecular Combing will be performed in central labs. All histological data will be analyzed by a central reading.

So far, one clinical site is active, and the first patient has been enrolled in June 2016. To date, 300 patients were enrolled, and about 65% of them are HR-HPV positive.

An interim analysis planned after 6 months evaluated 126 patients.

Conclusion
The EXPL-HPV-002 study will evaluate the diagnostic and prognostic values of HR-HPV integration status detected by Molecular Combing. Integration can prove to be a reliable biomarker that can specifically differentiate between women with a high risk from women with a low risk of developing cervical precancerous lesions or cancer. While the first will require immediate treatment, the other will require appropriate monitoring. Molecular Combing technology, as well as the first results of the interim analysis will be presented.
FC 22-02
HPV-16 variant´s and IGF1R overexpression induces resistance to radiotherapy in uterine cervical cancer

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Background / Objectives

Several causes of the variable radiotherapy (RT) efficacy have been studied without convincing results. If the HPV-16 infection has been hypothesized to be a predictor of poor response to RT (Ferdousi et al., 2010, Moreno-Acosta et al., 2017), the real clinical impact of HPV-16 and its prognosis significance is still to be demonstrated. In the recent studies (Zacapala-Gómez et al., 2016), was reported that factor receptor insulin-like growth1 (IGF1R) is over-expressed by effect of E6 AA-a, and E-G350 HPV-16 variants. Previous studies have shown a role for IGF1R in cellular radio-resistance in cervical carcinoma; Moreno-Acosta et al., 2012, found that the over-expression of IGF1R is a predictive marker for patients (HPV16 (+)) undergoing Radiotherapy because overexpression of this receptor confers 28.6 times greater risk of treatment failure. The aim of the present study was to prospectively report the detection of HPV-16 variants, gene expression IGF1R and assess the relationship with treatment response.

Methods

Detection of HPV 16 variants of 19 patients by PCR-SSCP and direct sequencing and analysis of IGF1R gene expression by real-time PCR. Of these patients, 15 underwent exclusive radiotherapy and four underwent radiochemotherapy.

Results

Three months after treatment completion, out of the 15 patients receiving exclusive RT, 8 experienced complete responses: 3 with the European T350 variant (E-T350 and IGF1R low expression), 2 with the European G350 variant (E-G350) and IGF1R negative expression), 2 with an undetermined European variant (E-Nd) and IGF1R negative expression), and 1 with an Asian-American variant (AAa) and IGF1R negative expression. The other 7 experienced no complete response: Three patients were diagnosed a partial response (2 E-T350, 1 E-G350, and IGF1R overexpression), 3 had a stable tumor (2 E-G350, 1 E-Nd and IGF1R overexpression) and 1 experienced tumor progression (AAa and IGF1R overexpression).

Conclusion
The presence of E-G350 and non-european (eg. AA) variants and overexpression of IGF1R in the no complete response group could be related with radioresistance. Larger prospective trials are needed to validate the presence of HPV-16 variants and IGF1R expression as a biomarkers of radioresistance.

References


Effects of HPV16 E6 and E7 oncogenes on genomic stability in HCT116 cells

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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. The effects of HPV16 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 instable. 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 HPV16 E6 and E7.

Methods

Western Blotting and RT-qPCR were performed to characterize HPV16 E6 and E7 expression in selected doxycycline inducible HCT116 clones. Effects on centrosome numbers and spindle poles formation during mitosis were analyzed using gamma-tubulin immunostainings. DNA damage in HCT116 clones induced for E6 and E7 expression was evaluated by staining of the phosphorylated histone component γH2AX, a marker for DNA double strand breaks. The number of aneuploid cells in response to HPV 16 E6 and E7 expression was determined by propidium iodide staining of the DNA and subsequent FACS analysis.

Results

Induction of both oncogenes elevated the number of interphase cells showing abnormal centrosome numbers. Additionally, the percentage of cells with 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. As a result of the deregulated distribution of chromosomes during mitosis, E6- and E7-expressing cells showed increased rates of DNA damage and aneuploidy.

Conclusion

In conclusion, HPV16 E6 and E7 induce genomic instability in HCT116 cells as indicated by abnormal spindle pole formation and increased DNA damage rates.
Subsequent analyses on gene copy number variations and differential methylation patterns will also help to understand how and how fast genomic stability is affected by the HPV oncogenes, deepening the insight into mechanisms and causes promoting malignant progression to cervical cancer.
Staging of Cervical Pre-Cancer using single cell mRNA E6/E7 and cell cycle (OncoTect 3DX)

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Background / Objectives

New therapeutics directed at treating pre-cervical cancer changes prior to the development of cervical cancer require staging 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 lo, post-G0/G1% hi. The ability to stratify cervical cancer abnormalities in an automated, high-throughput manner is advantageous for companion diagnostic applications.
CELL ADHESION AND CELL-CELL SIGNALLING ARE AFFECTED BY HPV INTEGRATION, WHILE DEREGLATION OF SPECIFIC PATHWAYS OCCUR DURING THE CIN3 TO CERVICAL CANCER TRANSITION

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Background / Objectives

Both HPV-positive and negative cervical cancers are associated with cell cycle deregulation, but the actual molecular events, remain elusive. To this end, we employed in vitro and in vivo models of cervical cancer by i) investigating the genomic and transcriptomic effects of the presence or absence of HPV in four informative cervical cell lines, and ii) validating these transcriptomic patterns in tissues from patients with normal, CIN3 and cervical cancer stages.

Methods

Whole exome sequencing and RNA sequencing were performed in a normal cervical cell line (HCK1T), in one HPV (−) C33A, and in two HPV (+) cell lines, HeLa [HPV18+] and SiHa [HPV16+], and in 12 samples of normal, CIN3 and cancer stages.

Results

RNA-sequencing revealed the main integration sites of HPV18 and HPV16 in chromosomes 8 and 13, respectively. Furthermore, a total of 212 genes (85 upregulated and 127 downregulated) were differentially expressed in HeLa and SiHa only. The majority of the downregulated genes are involved in processes of cell adhesion, cell-cell signaling and differentiation. The upregulated genes are involved in embryonic morphogenesis, apoptosis, cell cycle and in positive regulation of transcription. Whole exome sequencing revealed that 1,257 genes were mutated in HeLa and SiHa cells only, consistent with their expression profiles affecting processes of cell adhesion and development. Sixteen of these mutated genes were also differentially expressed between HPV [+] and HPV [-] cells. We validated these data in clinical samples from normal, CIN3 and cervical cancer tissues. The CIN3 and cervical cancer transcriptomes exhibited minimal similarities. With more than 2,000 transcripts differentially expressed among CIN3, cervical cancer and normal tissues, <400 genes showed similar expression patterns. Chemotaxis and immune-related processes were downregulated in CIN3 patients, while cell cycle and mitosis
were upregulated only in cervical cancer patients. Consistent with this, most of the transcriptional regulators controlling the immune and defense response, several cytokines and interferon regulatory factors, were downregulated, while in cervical cancer, regulators controlling cell cycle progression, were upregulated. Interestingly, many pluripotency-related genes displayed elevated gene expression only in CIN3, suggesting the establishment of a transient pluripotency-like phenotype.

**Conclusion**

These combined data imply that the presence of HPV in cervical cells initially leads to aberrant expression of genes controlling cell-cell signaling and cell adhesion, while at the precancerous stages, distinct genes and pathways are deregulated during the transition from CIN3 to cervical cancer.
DISCOVERY OF BIOMARKERS FOR IN VIVO IMAGING OF CERVICAL PRECANCERS IN THE STUDY TO UNDERSTAND CERVICAL CANCER EARLY ENDPOINTS AND DETERMINANTS

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Background / Objectives

Cervical cancer is the second-leading cause of cancer death in women globally, with a disproportionate burden on developing countries. Human papilloma virus (HPV) vaccination will not eliminate cervical cancer in the short term, so screening programs will remain essential for decades. HPV DNA testing is a highly sensitive screening method now being implemented in more settings, but its low specificity mandates triage (secondary) testing for positively screened women to avoid overtreatment harms which may include adverse pregnancy and fertility outcomes. Currently, triage options in low income settings are limited. Specific biomarkers that could be readily detected during the patient encounter through in vivo imaging present a novel promising triage strategy. The discovery of membrane biomarkers of cervical cancer and precancerous lesions may therefore enable the development of specific, sensitive, low cost in vivo detection tests for prevalent precancers.

Methods

The Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED), which includes approximately 3000 women referred for colposcopy from 2003 to 2011 with abnormal screening results, was designed to investigate cervical carcinogenesis and improve the ability to predict which women with HPV infections and low-grade lesions will progress to cancer. Gene expression levels were determined from mRNA microarrays of SUCCEED tissue from 128 patients at all stages of progression to cervical cancer (1), and differential expression of genes across the spectrum of normal, cervical intraepithelial neoplasia (CIN), and cancerous tissues (alpha=0.05 for cancer vs normal expression and for CIN vs normal expression) was detected.
Results

Based on the above criteria, 48 genes encoding for proteins with membrane-bound Gene Ontology annotations that could be amenable to in vivo staining and visualization were identified. Nineteen genes were prioritized for further investigation according to plausibility of plasma membrane localization, altered expression during early carcinogenesis, cervical expression, antibody availability, and enzymatic activity, of which 15 had increased and four had decreased expression in CIN tissues.

Conclusion

Validation of candidate proteins through immunohistochemical staining of SUCCEED tissues is currently under way, which will be followed by the investigation of the in vivo imaging potential of validated candidates using both antibody-based and enzyme-activated optical imaging methods. Finally, promising candidates will be moved forward to clinical studies evaluating their clinical utility for triage to immediate treatment after positive cervical cancer screening results.

References

DETECTING CERVICAL CANCER VIA ELEVATED HPV ONCOPROTEINS E6/E7 – ACCURACY OF THE ONCOE6™ CERVICAL TEST

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Background / Objectives

Cervical cancer remains a major cause of cancer related mortality among women living in low and middle income countries (LMICs). A decrease in mortality has been achieved in regions where population wide screening was implemented, yet existing screening technologies have failed to reduce mortality in many LMICs. Reasons for the lack of effective implementation of cervical cancer screening in many regions in need are complex; the infrastructure needs (pathologist, complex instrumentation, cold chains requirements) of existing screening modalities and high false positive rate with regard to detection (and subsequent treatment) of true malignancy are contributing factors.

Methods

Cervical cancer is one of the very few cancers where the molecular cause can be pinpointed in nearly 100% of cases: elevated expression of the viral encoded E6/E7 oncoproteins as the consequence of “molecular accidents” allowing such deregulation. This knowledge suggested a very plausible and direct way to detect HPV-induced malignancy via detection of elevated E6/E7 oncoprotein levels. The OncoE6™ Cervical Test (“E6 Test”) has been developed to (i) achieve highest specificity by direct detection of a cancer causing agent, the HPV encoded oncoprotein E6, and to (ii) be robust, and easy of use; this, and the simple training requirements allow implementation in virtually any setting. The E6 Test has been used in studies in many LMIC settings worldwide, and it has consistently revealed high clinical specificity (~ 99%) and positive predictive value for CIN3+. In several instances, the E6 test detected high-grade cervical disease where traditional methods (cytology, colposcopy) have failed to do so.
Conclusion

We will present a synopsis of the clinical accuracy of the E6 Test in a variety of real world settings, including studies on HPV driven oropharyngeal cancer, and we will critically discuss typical use scenarios for the E6 Test.
FC 22-08
Genome-wide microRNA profiling of hrHPV-positive self-samples: Promising triage markers for early detection of cervical cancer

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Background / Objectives

The effectiveness of cervical screening programs is hampered by suboptimal participation rates. Offering cervicovaginal self-sampling for high-risk HPV (hrHPV) testing has been shown to increase the participation. Since only a minority of hrHPV-positive women is at risk of cervical cancer, further stratification (triage) is needed to avoid overtreatment. MicroRNAs (miRNAs) represent a potential class of triage markers and their deregulation has been implicated in cervical cancer. At present, little is known about genome-wide miRNA expression patterns in cervical precancerous lesions (CIN3) and, most importantly, it is unknown whether deregulated miRNA expression is detectable in self-samples. In this study we set out to determine genome-wide miRNA profiles in hrHPV-positive self-samples in order to identify miRNAs detectable in self-samples that can predict the presence of CIN3 and cervical cancer.

Methods

Small RNA sequencing (sRNA-Seq) was conducted to determine genome-wide miRNA expression profiles in 77 hrHPV-positive self-samples (36 of women without cervical disease during follow-up (≤CIN1), 37 of women with CIN3 lesions, and 4 of women with squamous cervical carcinomas (SCC)). Logistic regression analysis was performed to identify the best miRNA panel with the highest combined sensitivity and specificity for CIN3 detection. Candidate miRNAs were validated by qPCR in an independent cohort of 164 hrHPV-positive self-samples (101 ≤CIN1, 49 CIN3, 14 cervical cancers).

Results

Classification of sRNA-Seq data resulted in the identification of 8 differentially expressed miRNAs with an area under the curve (AUC) of 0.89 for CIN3 detection. Six out of eight miRNAs could be validated in an independent self-sample series by qPCR, showing that CIN3 and cervical cancer associated miRNAs can be detected in hrHPV-positive self-samples.

Conclusion
This study is the first to determine genome-wide miRNA profiles in self-samples and reveals that miRNA expression analysis offers a promising novel molecular strategy for CIN3 and cervical cancer detection in hrHPV-positive self-samples. Moreover, our small RNA-Seq data will lead to a better understanding of the contribution of miRNA diversification in cervical carcinogenesis.
Prognosis of donor patients according to the characteristics of Patients derived xenograft (PDX) tumor in gynecological cancer.

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Background / Objectives

Patients derived xenograft (PDX) reflects molecular and cellular characteristics of the donor tumor and is an important model in the study of cancer biology. PDX models also have been developed to apply more personalized strategy against cancer, such as evaluation of drug efficacy and biomarker validation. The purpose of this study is to evaluate the prognosis of donor patients according to the characteristics of PDX tumor in gynecological cancer.

Methods

Cancer tissues from gynecological cancer patients (ovary, cervix and uterine cancer; total 107 cases) were fragmentized to 3 mm pieces and transplanted into athymic nude mice. The volume of each PDX tumor were measured using a digital caliper for up to 1 year and 4 months after transplantation [(Short x Short x Long) / 2 = vol. (mm3)]. The largest PDX tumor in each patient's cases were selected, thereafter, engraftment and growth rate were calculated in PDX tumor (Success of implantation is a confirmed growth-up in PDX tumors; Growth rate is slope value of growth equation per PDX). Donor patient’s prognosis were evaluated by survival rate calculated using survival significance methods, applied with the previously reported cut-off value (Finder et al, PLoS One. 2012;7(12):e51862.).

Results

Engraftment results showed that implantation succeeded PDX tumor cases had a tendency of poor prognosis than failed cases in 5 year disease free survival, (Log rank p = 0.2446, HR 3.246, 95% CI = 0.6876 to 15.33). As for the growth of PDX tumors, fast growing cases showed a tendency of poor prognosis in 5 year disease free survival (Log rank p = 0.1562, HR = 2.982, 95% CI = 0.4712 to 18.87) and showed a significant poor prognosis in 5 year overall survival (Log rank p = 0.0374) than slow growing PDX tumors.

Conclusion

This study reveals concordance of aggressive cancer biology, with fast growing in PDX tumors and poor prognosis of the donor patient. These findings may be an important resource for studying cancer biology and supporting PDX model for developing personalized strategy against cancer.
References

Key words: Patients derived xenograft (PDX); Gynecological cancer; Prognosis;
META-ANALYSIS ON THE PROGNOSTIC SIGNIFICANCE OF P16INK4A AND HPV DNA IN ANAL SQUAMOUS CELL CARCINOMAS

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Background / Objectives

Anal squamous cell carcinomas (ASCC) represent the most common histologic entity among anal cancers. Oncogenic human papillomavirus (HPV) types play an etiological role in a large proportion of ASCC as indicated by the detection of HPV DNA in up to 90% of cases frequently accompanied by overexpression of the cell cycle regulator protein p16INK4A on immunohistochemistry (IHC). By analogy to head and neck squamous cell carcinomas, it has been suggested that HPV DNA and p16INK4A status might be of prognostic relevance in ASCC patients. However, the reported survival rates for ASCC patients, stratified by these two markers, differ among the published studies.

We aimed to determine the prognostic relevance of oncogenic HPV DNA and p16INK4A status among all published literature in a systematic review and meta-analysis.

Methods

A broad search string was designed to identify all published studies analyzing p16INK4A expression by IHC and providing survival data in patients diagnosed with ASCC. Overall survival (OS) was analyzed performing Cox Regression including p16INK4A IHC, HPV DNA status and clinical data as covariates. Authors were contacted to obtain lacking information or data.

Results
16 studies were found to be eligible for inclusion in the final analysis. From 8 of them we obtained the individual patient records comprising 666 ASSC cases. 84.3% of 555 ASSC tested positive for oncogenic HPV DNA. 81.8% of 658 ASSC demonstrated overexpression of p16\textsubscript{INK4A} on IHC. Patients with ASSC demonstrating p16\textsubscript{INK4A} overexpression had a significantly longer median OS than patients without p16\textsubscript{INK4A} overexpression (36 vs. 28 months (m), respectively), hazard ratio (HR)=0.42 (95% confidence interval (CI), 0.30-0.61) in a pooled analysis. Patients with HPV DNA-positive ASSC also demonstrated a significantly better median OS compared to HPV DNA-negative ASSC patients (39 vs. 26 m, respectively), HR=0.39 (95% CI, 0.27-0.57). Hazard ratios for gender (female vs. male), T-stage (T3/4 vs. T1/2), N-stage (N+ vs. N0), M-stage (M+ vs. M0), HIV status (positive vs. negative) and age were 0.42 (95% CI, 0.30-0.59), HR=2.88 (95% CI, 2.06-4.04), HR=1.92 (95% CI, 1.37-2.70), HR=3.29 (95% CI, 1.60-6.02), HR=1.30 (95% CI, 0.66-2.33), HR=1.02 (95% CI, 1.01-1.03), respectively.

**Conclusion**

p16\textsubscript{INK4A} overexpression and oncogenic HPV DNA are detected in a large proportion of ASSC patients and predict better survival compared to p16\textsubscript{INK4A} or oncogenic HPV DNA-negative ASSC patients. The obtained data will be further analyzed in multivariate analyses. In the future, we will use digitized Kaplan-Meiers to meta-analyze the 16 studies and use available individual patient data from 8 studies to validate statistical methods.
VAGINAL AND ANAL HRHPV INFECTION AMONG FEMALE SEX WORKERS IN AMSTERDAM, THE NETHERLANDS: PREVALENCE AND CONCORDANCE


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Background / Objectives

Condom use is high among female sex workers (FSW) in Amsterdam, but because of limited condom induced protection against human papillomavirus (HPV) infection, FSW may be still at high risk of HPV infection and HPV-related diseases. We aimed to study risk factors, prevalence and concordance of genital and anal high-risk (hr) HPV infection among FSW in Amsterdam.

Methods

In 2016, FSW aged ≥18 years having a consultation regarding sexually transmitted infections (STI) with the Prostitution and Health Center (PG292) in Amsterdam were invited to participate. Participation entailed taking a vaginal and anal self-swab. Demographics and sexual behavior data were collected in the consultation and HPV DNA was analyzed using SPF10-PCR-DEIA-LiPA25-system, version 1. Uni- and multivariable logistic regression analyses were performed to assess determinants of type-specific vaginal and anal hrHPV infection. Determinants of vaginal and anal hrHPV infection were uni- and multivariably assessed using logistic regression with generalized estimating equations (GEE).

Results

We included 304 FSW with a median age of 29 years (IQR 25-37). The STI prevalence at moment of inclusion was 9% and the prevalence of vaginal and anal hrHPV among participants was 46% and 55%, respectively. The most prevalent vaginal hrHPV infections were types 31 (11.8%), 52 (10.2%), 51 (8.6%), and 16 (8.2%). The most prevalent anal hrHPV infections were types 51 (14.1%), 31 (12.8%), 16 (11.5%) and 18 (12.2%). The highest concordance between vaginal and anal infections was found in types 31 (5.6%), 52 (4.6%), 18 (4.3%) and 16 (3.9%). A risk factor for both vaginal and anal hrHPV was opposite anatomical site of infection (OR 1.32, 95% CI 1.24-1.40; OR 1.40, 95% CI 1.31-1.50, respectively). Additionally, a risk factor for vaginal hrHPV was region of birth (Eastern Europe OR 0.95, 95% CI 0.93-0.97; America's OR 0.96, 95% CI 0.94-0.99; other regions OR 0.95, 95% CI 0.93-0.98; compared to the Netherlands).
Conclusion

Vaginal and anal hrHPV prevalence is high among FSW in Amsterdam, the Netherlands. Even in multivariable logistic regression using GEE, concordance between vaginal and anal type-specific hrHPV infections was high.
REDUCTION IN SEXUAL ACTIVITY FOLLOWING A DIAGNOSIS OF ANAL HIGH-GRADE INTRAEPITHELIAL LESION (HSIL) AMONG GAY AND BISEXUAL MEN (GBM)


Background / Objectives

To assess the impact of a diagnosis of anal HSIL on subsequent sexual activity in GBM.

Methods

The Study of the Prevention of Anal Cancer (SPANC) enrolled GBM in Sydney who had never previously undergone high-resolution anoscopy (HRA). At baseline and 6-month visits, a behavioural interview, cytological ± histological assessments were performed. We examined the association between a baseline HSIL diagnosis and subsequent changes in sexual behaviour.

Results

Among 617 GBM enrolled (median age 49 years; 35.7% HIV-positive), 518 (84%) attended 6-month follow-up. The number of participants reporting any recent casual sex declined among 232 (37.6%) men diagnosed with HSIL (76.8% to 67.7%, p=0.050) but not in those negative for any squamous intraepithelial lesion (SIL) at baseline (76.0% to 70.1%, p=0.239). There was also a reduction in median partner numbers in the previous 6-months among men with HSIL (9 vs 5, p=0.048) but not among SIL-negative men (7 vs 5.5, p=0.128). Among both men with HSIL and men without SIL at baseline, the number of episodes of receptive penile-anal sex, rimming, fingering, fisting and toys remained unchanged. Neither presence nor duration of pain or bleeding following baseline HRA were associated with subsequent reduction in partner numbers.

Conclusion
GBM in SPANC reduced casual sexual contact and partner numbers following a diagnosis of HSIL. This could be a conscious decision based on a perception of reducing future risk of anal cancer or a fear of transmitting the causative high-risk HPV infection to partners. Further investigation of the reasons behind this change in sexual activity is warranted.
PREDICTORS OF 12-MONTH PERSISTENT HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL) IN A COHORT OF GAY AND BISEXUAL MEN

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Background / Objectives

Gay and bisexual men (GBM), particularly HIV positive GBM, are at greatly increased risk of anal cancer. The anal cancer precursor HSIL is so highly prevalent in GBM that it is clear most HSIL do not progress to cancer. We examined predictors for 12-month HSIL persistence and clearance in GBM with HSIL at study baseline, with the aim of identifying clinically-useful predictors of risk of progression to anal cancer.

Methods

Participants were 617 GBM from the ongoing Study of the Prevention of Anal Cancer conducted in Sydney, Australia. They completed detailed demographic and behavioural questionnaires and underwent cytological, histological and HPV assessments of anal canal samples at baseline, 6- and 12-months. Composite HSIL was defined as either cytological and/or histological detection. Among those with HSIL at baseline, clearance and persistence were defined by non-detection (double negative) and persistent (double positive) detection of HSIL at both 6- and 12-month visits, respectively.

Results

By March 2017, 485 participants had completed their 12-month visits and of these 435 (89.7%) attended all three of the baseline, 6- and 12-month visits. A total of 390 men (63.2%) had both cytological and high resolution anoscopy results available.
from each visit. Of these, the median age was 49 years and 137 (35.1%) were HIV-positive. Among 159 (40.8%) who had composite HSIL at baseline, 44 (27.7%) had HSIL detected at baseline only and 89 (56.0%) had HSIL which persisted at all three visits. HSIL in older men was much less likely to clear (p=0.005) than in younger men. HIV status was not associated with HSIL clearance. HSIL-AIN3 lesions were half as likely to clear as HSIL-AIN2 lesions (HR 0.42, 95% CI 0.20-0.85, p=0.016). Larger lesions (more than one octant) were also less likely to clear (HR 0.33, 95% CI 0.009-1.25, p=0.005). HPV16 positivity at baseline was strongly associated with decreased rates of clearance of HSIL (RR=0.15, 95% CI 0.006-0.36). Clearance was lowest in those who had HPV16 (p<0.001) and type-specific non-HPV16 high risk HPV (p<0.001) at both 6- and 12-month visits.

**Conclusion**

Among men with HSIL at baseline, HSIL persisted for at least 12 months in over half the participants and one in five had no evidence of HSIL at two subsequent visits. Both baseline and persistent high risk HPV and in particular HPV16, strongly predicted lack of clearance of HSIL. Two HPV tests separated by at least 6 months may identify a subgroup of men with HSIL that is likely to be persistent, and thus at high risk of progression to cancer.
Baseline low- and high-risk HPV prevalence in rectal swabs from men prior to selective immunisation with the quadrivalent HPV vaccine in Scotland

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Background / Objectives

The quadrivalent vaccine prevents infection with HPV types 6, 11, 16 and 18 and has been shown to induce strong and sustained neutralising antibody responses that confer protection against infection and associated disease. Such data stem from population-based surveillance of women who have largely been part of catch-up cohorts from a school-based programme. We aimed to collect baseline data on rectal HPV prevalence from a cohort of men who attended sexual health services, prior to implementation of a selective immunisation programme for men-who-have-sex-with-men (MSM) up to age 45.

Methods

Approximately 1200 rectal swabs were obtained from males attending for sexual health services in Edinburgh, Scotland. Swabs had originally been collected for (routinely indicated) Chlamydia trachomatis testing. Residual material was subject to molecular HPV genotyping using automated extraction and a luminex-based assay which detects 24 HPV types including all established high-risk types and all those included in the quadrivalent vaccine. At time of abstract preparation, results are available for 1064 samples.

Results

HPV prevalence in this population was high; 782/1064 (73%) were HPV positive and 531/1064 (50%) were positive for at least 1 of the types within the quadrivalent vaccine. When vaccine types were counted individually (including as part of a mixed infection) HPV 6, 11, 16, and 18 accounted for 156, 74, 362, and 80 infections respectively. Of those positive for at least 1 of the 4 vaccine types, none were positive for all 4 types.

Conclusion

These preliminary data indicate that the quadrivalent vaccine has the potential to have a significant impact on the prevalence of HPV in this population given that 50% are infected with one of the types included in the quadrivalent vaccine. Comprehensive data which stratify HPV status by age and HIV status will also be presented.

INCREASED RISK OF HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA (HGAIN) IN PATIENTS WITH ANAL WARTS ASSOCIATED WITH HERPES SIMPLEX TYPE 2, GONORRHOEA AND OTHER STI’S

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Background / Objectives

Anal cancer rates are rising in men and women worldwide with anal intraepithelial neoplasia (IN) thought to be a precursor. Anal warts have been associated with high rates of IN especially in HIV infection, and anal cancer has been recognised as arising from anal warts. Cofactors other than HPV are likely to be associated with the development of anal cancer and its precursors. A surgical data base with epidemiological and histological data from patients surgically treated for anal warts was created at Royal Perth Hospital in 1996, and findings from this database have been previously reported.1,2

Methods

Patients who underwent surgical excision of anal and/or perianal condylomata acuminata or mapping biopsies from December 1995 to November 2016 were included in the analysis. Demographic data were collected including sex, sexual preference, lifetime sexual partners, history of gonorrhoea, or chlamydia and serological data for syphilis, herpes type 2 antibody (HSV2) and HIV 1 and 2 antibody at the time they were enrolled for surgery. Anal HPV testing by Digene Hybrid Capture II for high-risk strains (hrHPV) was included as a standard of care from June 2005 onwards.

Results

463 patients were included in the analysis, the majority of whom were MSM (367). Almost one third were HIV positive and had high-grade squamous intraepithelial neoplasia (HSIL). Overall 75% of the samples tested positive for hrHPV, with HIV positive men having 95% hrHPV. HSV-2, gonorrhoea, and syphilis were associated with the risk of HGAIN: OR 14.3 (95%CI 6.20-33.1), OR 10.4 (95%CI 4.32-25.0) OR 9.83 (95%CI 4.20-23.0) respectively.

Conclusion
The association of STI's such as gonorrhoea, syphilis and genital herpes with the presence of HGAIN deserves further study. Given rates of gonorrhoea are increasing in the MSM community, patients should be counselled to avoid STI acquisition and use condoms to reduce their risk of anal cancer. Detection of HGAIN is problematic with high resolution anoscopy clinics being scarce. A history of gonorrhoea, syphilis or HSV-2 could be used to triage patients at risk of HGAIN to these clinics.

References


Background / Objectives

Gay and bisexual men (GBM) are at increased risk of HPV-associated anal cancer. Screening for the precursor high-grade squamous intraepithelial lesions (HSIL) analogous to cervical cytology screening has been proposed but there is currently no agreed algorithm. Anal HSIL is highly prevalent in GBM, however it is clear that most anal HSIL do not progress to cancer. Identification of biomarkers to establish patients at highest risk of cancer are therefore needed. Change in DNA methylation is recognised as an early essential step in carcinogenesis, and is predictive of cervical HSIL and cancer. It is thought that DNA methylation patterns may also predict anal HSIL. Here, DNA methylation was evaluated as a potential tool to identify persistent anal HSIL among GBM.

Methods

Samples were obtained as part of the Study of the Prevention of Anal Cancer, a 3-year natural history study of anal HPV and related disease in GBM aged 35 years and older. HSIL was defined as a composite of cytology and histology diagnoses (ie had one or both cytological possible HSIL (pHSIL) and/or histological HSIL). Persistent HSIL was defined as HSIL detected at all of baseline, 6 and 12 months clinic visits. DNA was extracted from anal cytology PreservCyt samples from the baseline clinic visit, and subjected to bisulfite conversion. Methylation-specific
quantitative PCR (msqPCR) on promoter regions of CADM1, MAL and miR-124-2 genes were performed, with the positive threshold for each marker set at 1.5%, 0.5% and 0.5% methylation, respectively. Sensitivity and specificity of each marker for detecting prevalent baseline and persistent HSIL were calculated.

Results

Of the 165 participants included, 52 (31.5%) were HIV-positive and 69 (41.8%) had composite HSIL at baseline. In total, 23 (13.9%) had persistent HSIL at 12 months post-baseline. Sensitivity and specificity, positive and negative predictive value (PPV, NPV) of each methylation marker are shown in the table.

<table>
<thead>
<tr>
<th>Marker</th>
<th>HSIL diagnosis</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAL</td>
<td>Baseline</td>
<td>62 (50-74)</td>
<td>41 (31-52)</td>
<td>43 (34-54)</td>
<td>60 (47-72)</td>
</tr>
<tr>
<td>MAL</td>
<td>Persistent</td>
<td>74 (52-90)</td>
<td>42 (33-51)</td>
<td>18 (11-28)</td>
<td>90 (80-96)</td>
</tr>
<tr>
<td>mi-R-124-2</td>
<td>Baseline</td>
<td>52 (40-64)</td>
<td>41 (31-52)</td>
<td>39 (29-50)</td>
<td>54 (42-66)</td>
</tr>
<tr>
<td>mi-R-124-2</td>
<td>Persistent</td>
<td>57 (35-77)</td>
<td>42 (33-51)</td>
<td>15 (8-24)</td>
<td>85 (74-92)</td>
</tr>
<tr>
<td>CADM1</td>
<td>Baseline</td>
<td>36 (25-49)</td>
<td>73 (63-81)</td>
<td>49 (35-63)</td>
<td>61 (51-70)</td>
</tr>
<tr>
<td>CADM1</td>
<td>Persistent</td>
<td>48 (27-69)</td>
<td>70 (62-78)</td>
<td>22 (12-36)</td>
<td>88 (81-94)</td>
</tr>
</tbody>
</table>

Conclusion

Methylation markers MAL, CADM1 and miR-124-2 in baseline anal swab DNA may have higher sensitivity and similar specificity for the detection of persistent HSIL than for the detection of baseline HSIL, however larger numbers and further studies are needed to evaluate markers for detection of persistent HSIL.
Is the persisting HPV genotype on anal swab the causative genotype in HGAIN lesions?


Background / Objectives

To study the correlation between persistent type-specific HPV infection on anal swabs and the causative HPV type in HGAIN lesions in HIV+ MSM.

Methods

A cohort of HIV+ MSM was followed for two years with 6-monthly anal swabs. Swabs were tested for HPV DNA and typed using the SPF10-PCR-DEIA-LiPA25v1 system. Men in whom at least 4/5 anal swabs were positive for the same HPV type were considered to have a persistent anal HPV infection. After this 24-month period men were assessed by High Resolution Anoscopy (HRA), and biopsies were taken. For the present analysis, we selected MSM with persisting anal HPV infections on swab and HGAIN on histology (N=30). After reviewing of HE and p16 slides for the worst diagnosis (AIN2 or AIN3), worst lesions collected using laser capture microdissection were tested for HPV with the same system which resulted in identification of the causative genotypes. We assessed the correlation between persistent type-specific HPV infection on swab and the causative HPV type of HGAIN lesions.

Results

After exclusion of 5 patients in whom the worst diagnosis upon re-examination was less than HGAIN, 51 biopsies from 25 men remained. Worst lesion found on patient level was AIN2 in 9 men and AIN3 in 16 men. On the anal swabs, 12 men had persisting HPV infection of a single HPV genotype (3 lrHPV, 9 hrHPV), and 13 with multiple types. Of the men with multiple persisting HPV genotypes, one man had lrHPV genotypes only, 4 had hrHPV genotypes only and 8 had both lrHPV and hrHPV genotypes. HPV6 was the most frequently found persisting genotype (36%). On biopsy, four men had (multiple) HGAIN lesions with different HPV genotypes and 21 men had one HGAIN lesion with a single causative HPV genotype. The most
frequently found genotype in HGAIN lesions was HPV 16 (28%, 7/25). The causative HPV type (1 lrHPV and 24hrHPV) was the same as the persisting type in 11/25 (44%) men with HGAIN: 2/9 (22%) in AIN2 and 9/16 (56%) in AIN3. In 8/25 men (32%) the causative type was not persistent, but was detected in at least one of the swabs. In 6/25 men the causative type was never found in anal swabs (24%).

Conclusion

A persisting genotype was marked as a causative genotype in only 44% of the men with a HGAIN lesion, with a remarkable difference in correlation with AIN2 (22%) and AIN3 (56%). In 32%, the causative genotype was detected in at least one of the swabs and in 24% the causative genotype was never detected on swab in the 2 years prior to HRA. These results demonstrate that the causative genotype is often not persistently or not detected on swabs. Therefore, serial anal swabs may have limited value in screening for HGAIN, but AIN3 is linked to persistence more often than AIN2.
FC08-09
HPV TESTING USING XPERT HPV ON SELF-COLLECTED VAGINAL SWABS VS. CLINICIAN-COLLECTED CERVICAL SAMPLES

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Background / Objectives

HPV testing of self-collected vaginal swabs may help expand access to cervical cancer screening in low-resource settings. We compared the utility for screening of Xpert HPV when run on self-collected vaginal swabs vs. clinician-collected cervical samples.

Methods

At a colposcopy and a primary care site in Cape Town, South Africa, 585 HIV-negative aged 30-65 years were recruited. Self-collected vaginal swabs and clinician-collected cervical samples were tested using Xpert HPV. This assay detects high risk HPV in 5 channels: HPV16, HPV18,45, HPV31,33,35,52,58, HPV51,59, HPV39,56,66,68. Outcome of cervical intraepithelial neoplasia grade 2/3 or cancer (CIN2+) was determined by colposcopy and histology for all women.

Results

Sensitivity of Xpert HPV to detect CIN2+ was similar in self- (85.7%) and clinician- (88.3%) collected samples, but specificity was lower in self- (77.0%) vs. clinician- (87.3%) collected samples. Sensitivity could be retained at high levels if screen-positive was defined as positivity for one or more of the three channels detecting HPV 16,18,45,31,33,35,52,58 (84.4% vs 87.0% self vs. clinician, respectively). Restricting to these channels, specificity improved to (82.3 vs 90.5% self- vs. clinician, respectively). Defining screen-positive based on more stringent cycle thresholds (Ct) and allowing sensitivity to be 80%, resulted in specificities of 87.3% and 94.7% in self- vs. clinician-collected samples. If a second clinician-collected sample is obtained and tested from self test-positive women, at 80% sensitivity, specificity can be improved to 92.9%.

Conclusion

HPV testing on self-collected samples has excellent sensitivity. Specificity can be improved by HPV type selection and more stringent Ct cut-offs. Specificity can be improved further with secondary triage with HPV testing on clinician-collected samples.
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HPV16 MINORITY VARIANTS AMONG CERVICAL AND ANAL SAMPLES WITH SINGLE HPV16 OR MULTIPLE HPV TYPES INFECTIONS

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Background / Objectives

To assess presence of minority variants (MV) in HPV16 genomes isolated from clinical cervical and anal specimens of patients followed in Paris area, France, using ultra-deep sequencing of the whole viral genome.

Methods

HPV detection and typing was performed with Anyplex®II HPV28 detection kit (Seegene). Ultra-deep sequencing was performed using Illumina® platform. HPV16 lineages were determined by phylogenetic analysis using RAxML. HPV16 viral loads were determined by “in house” real-time PCR assays. Viral genome integration ratio was assessed by E2/E6 ratio.

Results

We assessed 44 consecutive smears routine samples (28 cervical and 16 anal [n=12 men]) positive for HPV16. 19 patients (43%) were HIV-infected. Cytohistologic data showed 10 LSIL and 5 HSIL in cervical samples, 3 LSIL and 8 HSIL in anal samples. Overall, multiple HPV (mHPV) infections (high risk [hr] and/or low risk [lr]) were observed in 32 samples (73%). In anal samples mHPV infections were present in a higher proportion (94% vs 61%, p=0.03) and with a higher diversity of hrHPV types (p=0.04) than in cervical samples. Phylogenetic analysis showed diversity in HPV16 lineage variants with 32 A lineage (73%), 3 B (7%), 7 C (16%) and 2 D (4%). Ultra-deep sequencing analysis revealed that nucleotidic non synonymous substitutions
were present in minority proportion (i.e. ranging from 2 to 20%) in 14 samples (32%),
without difference between cervical and anal samples (32% and 31%, respectively).
Viral MV were present at the median proportion of 3.4% (IQR=2.4-6.4). Most of MV
(64%) presented only a single nucleotide variation on the whole genome, located in
E1 or E2 regions in most of cases (86%). Among cervical specimens, a lower
frequency of lrHPV coinfections was observed in samples displaying MV than in
samples without MV (11% vs 58%, p=0.04). A lower frequency of hrHPV coinfections
was also observed but was no significant (33% vs 63%). In addition, patients with
mHPV cervical infections had more frequently high HPV16 viral load (i.e. >50
copies/cell) than patients without mHPV infections (77% vs 20%, p=0.012). The
proportion of patients with a high integrated viral genome ratio (i.e. >50%) increased
with the cervical lesion grade: 44%, 56% and 80% in ASC-US, LSIL and HSIL,
respectively. Regarding anal samples, all except one had mHPV infections, 50%
were HSIL and 55% had a high integrated viral genome ratio.

Conclusion

This first large study of HPV16 whole genome ultra-deep sequencing showed
presence of viral MV in one third of the samples, in cervical as well as in anal
specimens. In addition, we evidenced that, in almost all cases, cervical samples with
MV had no lrHPV coinfections.
DETECTION OF CERVICAL HUMAN PAPILLOMAVIRUS IN WOMEN ATTENDING CERVICAL CANCER SCREENING BY VISUAL INSPECTION IN COTE D’IVOIRE


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Background / Objectives

Human papillomaviruses (HPV) cause precancerous lesions and cancers of the cervix. In Côte d’Ivoire, cervical cancer screening program based on visual inspection is the gold standard. This study aims to detect High risk (HR) HPV DNA on women attending for cervical cancer screening program based on visual inspection after application of acid acetic then lugol

Methods

From March to December 2015, endocervical samples from women attending cervical screening were tested for some HR-HPV. HPV DNA was amplified using PGM/Y09/11 primers which generated 450 base pairs at the L1 region. The samples harboring HPV DNA were genotyped using the multiplex PCR testing for HPV 16, 18, 31, 33, 35, 45 and 51 primers.

Results

The mean age of this population was 32 years. On 339 women enrolled on visual inspection 6.19% were positive. HPV DNA was obtained in 9.73 of the population. Thirty-one of 33 samples (93.93.%) of HPV DNA+ were genotyped using multiplex PCR testing for HPV 16, 18, 31, 33, 35, 45 and 51. Of those women with HPV DNA+. 28.57% had a single infection while 71.43% had a multiple infection. HPV genotypes prevalence were the followed: HPV 16 (30.00%), HPV 18 (25.00%), HPV 35 (20.00%), HPV 45 (20.00%), HPV 51 (3.30%) and HPV 33 (1.60%). By using PCR as gold standard VIA sensitivity was 16.12% and specificity 95.45 %

Conclusion

HPV prophylactic vaccine would prevent 33.33% of HR HPV infection with the 2v, 33.33% with the 4v and 66.66 % with the 9v vaccines respectively. In Cote d'Ivoire screening for cervical cancer with HR HPV testing and triaging for treatment with
visual inspection would represent a very efficient prevention of cervical cancer program.

References


DETECTION OF HIGH-RISK HPV DNA IN CHAGASIC MEGAESOPHAGUS WITH AND WITHOUT CANCER


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Background / Objectives

Esophageal cancer (EC) is the eighth most common type of cancer worldwide frequently found with esophageal squamous cell carcinoma (ESCC) differentiation. The main risk factors related to ESCC development are smoking, alcohol consumption, chagasic megaesophagus (CME) (common digestive chronic manifestation of Chagas Disease), and likely HPV, although the role of HPV in ESCC carcinogenesis is still disputable. Therefore, the present study aimed to detect high-risk HPV DNA in patients with chagasic megaesophagus with and without cancer and to correlate these findings with clinicopathological data.

Methods

Samples tissue/biopsy specimens fixed paraffin were retrospectively collected from the southeast region of Brazil obtained from patients treated in two hospitals: Universidade Federal do Triangulo Mineiro e Barretos Cancer Hospital. Cases were divided in two groups: CME (n=30) and ESCC/CME (n=21). The detection, and typing of high-risk HPV were performed by multiplex PCR (Luminex)

Results

Overall, the prevalence of HPV was higher in the CME group (n=15/30, 50%) when compared to the ESCC/CME group (n=8/21, 38%). Among the HPV types detected, HPV-16 and HPV-73 (13.3% and 6.7%, respectively) were detected with similar frequency in both groups. On the other, HPV-45, HPV-51 and HPV-56 (6.7%, 10% and 6.7%, respectively) were found only in the CME group, as well low-risk HPV-6 and HPV-11 (3.3% and 3.3%, respectively). In addition, HPV-positive patients (38.1%) had I/II (non-advanced) grade megaesophagus, whereas HPV-negative patients (61.9%) had grade III/IV (advanced), staging T3/T4 (91.7%), N0/N1 (91.7%) and M0 (75%). Regarding the pathological features of the ESCC/CME group, the most frequent type reported was moderately differentiated, which was more
frequently associated with HPV-negative status, yet not reaching statistical significance.

Conclusion

Despite the high frequency of HPV DNA detected in patients with chagasic megaesophagus with and without cancer, a statistically significant association was not found, thus further studies are important in order to understand the role of HPV in esophageal cancer in patients with megaesophagus.
P02-02
Comparative study of HPV prevalence in glans and urine between the patients with prostate cancer and benign prostatic hyperplasia


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Background / Objectives

Some recent studies demonstrate that the urinary tract is an alternative common site for HPV infection in men. Although urine is often used for investigating HPV infection in the urinary tract, the significance of HPV detected from urine samples has been not understood. We investigated the prevalence of human papillomavirus (HPV) in the genital and urinary tract among the patients with prostate cancer (Group A), ones with an elevated level of prostate specific antigen (PSA) with no evidence of prostate cancer (Group B), and ones with benign prostatic hyperplasia (BPH) without an elevated PSA (Group C). We compared HPV prevalence in glans and urine samples between the groups.

Methods

A total of 325 patients (42 cases in group A, 84 in group B, and 199 in group C) were enrolled in this study. Rubbed cells samples of glans and urine ones were collected from each patient, and sediment cells were preserved in liquid-based cytology solution, respectively. The β-globin gene was first amplified to confirm the adequacy of the extracted DNA in all samples. HPV-DNA and genotype was determined using GENOSEARCH-31 (BML, Co., Ltd., Nagoya, Japan).

Results

Mean age in group A, B, and C was 77.1, 68.2, and 72.7 years, respectively. Among the adequate samples, HPV was detected in 14.6% (6 cases), 16.6% (11 cases), and 26.8% (47 cases) of glans samples in group A, B, and C. HPV prevalence was higher in group C, but there were no significant differences between the groups. On the other hand, HPV prevalence in urine samples of group A, B, and C, was 12.5% (5 cases), 3.6% (3 cases), and 7.1% (13 cases), respectively. High-risk HPV was identified in 7.5% (3 cases) in group A, 3.6% (one case) in group B, and 3.8% in group C. In urine samples, there were also no significant differences in HPV prevalence between the groups, while high-risk HPV showed a significant higher prevalent in group A than in group B and C (p<0.05).

Conclusion

We found a higher prevalence in urine samples of the patients with prostate cancer, suggesting an interesting issue whether HPV infection in urinary tract can play any
roles in pathogenesis for men, especially in the development of tumors in the urinary tract.
HPV PREVALENCE 10 YEARS AFTER VACCINE INTRODUCTION IN GERMANY – DESIGN OF A POPULATION-BASED STUDY IN 20-25 YEAR-OLD WOMEN

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Background / Objectives

In Germany appx. 4,600 women are diagnosed every year with cervical cancer, leading to 1,500 deaths. Infections with sexually transmitted human papillomaviruses (HPV) are a prerequisite for cervical cancer. Since 2007, routine HPV vaccination for girls has been recommended in Germany. However, because HPV infections are not notifiable in Germany, other means of data collection are needed to evaluate and document the impact of HPV vaccination. In 2010-12, we conducted an initial population-based study among women aged 20-25 years to assess baseline HPV prevalence and genotype-distribution in a mainly vaccine-naïve population. To estimate HPV prevalence ten years after vaccine introduction and to assess possible effects of vaccination, a follow-up study will be carried out in 2017/18. Here we present details of the study design.

Methods

Nationwide population-based cross-sectional study to examine HPV prevalence in 20-25 year-old vaccinated and unvaccinated women in Germany. Cervico-vaginal self-sampling via EvalynBrush (Rovers, Netherlands) and multiplexed genotyping HPV test Optiplex (Diamex, Germany) is used.

Results

We will recruit at least 1,173 women aged 20-25 years. Recruitment will be based on a random sample from the residents’ registration offices of all communities in Germany, using a two-step sampling design stratified by geographical location (former Eastern/Western Germany) and population density (rural/urban). Using a self-sampling device participants will take vaginal cell samples that will be tested for HPV infections with 18 high-risk (e.g. 16, 18, 31, 45) and 8 low-risk types (e.g. 6, 11). In addition, participants will be asked to answer a questionnaire comprising questions on socio-demographics, sexual behavior, immunosuppressive diseases/medication, and HPV vaccination status; furthermore, they will be asked to provide proof of their vaccination status by uploading a photo of their vaccination card. The study was designed by considering major aspects of the baseline study to allow for comparison of results. First results of the study are expected in 2018.

Conclusion
The study will help to answer the following questions:

For women aged 20-25 years in Germany,

What is the prevalence of a) vaccine-preventable and non-vaccine-preventable and b) high-risk HPV types in 2017/18 and in comparison to the baseline study?
What is the difference in HPV prevalence in vaccinated vs non-vaccinated young women?
What is the effectiveness (at population level) of HPV vaccination?
What are risk factors for being unvaccinated or HPV positive?

The study will contribute to the evaluation of the existing HPV vaccination recommendation in Germany.
Trends in rates of treated RRP before and after HPV vaccination among New York children

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Background / Objectives

Recurrent respiratory papillomatosis (RRP) is a rare condition in children acquired around the time of delivery and is caused by HPV types 6 and 11 leading to wart-like growths in the respiratory tract resulting in hoarseness and airway obstruction. Its incidence in children is expected to decline with HPV vaccination which has been FDA-approved in the US since 2006. New York is a populous state with vaccination rates among girls typical of many states nationally, growing from percentages in the 50's to the 60's over the last decade (1). We expect changes in RRP incidence attributable to vaccination to first be evident in young children. We sought to determine whether the rate or frequency of treatments for RRP has changed since the approval of HPV vaccination.

Methods

We obtained data from NY State Ambulatory Surgery and Services Database (2) between years 2004-2013 for discharge encounters of children under the age of 18 who had ambulatory procedures with ICD-9 code corresponding to benign neoplasm of the larynx and procedure codes relating to typical RRP treatments. A visit linkage variable allowed for tracking patients who had multiple discharges in a year. All facilities licensed to perform same-day surgery in NY were included (as such, parameters rather than statistics were calculated). Unable to calculate RRP diagnosis with this source, we used treatment as a loose proxy for prevalence. Patients were placed into older (age 10-17) and younger (age <10) groups. Trends over time in number and age of patients treated per year and average number of treatments per patient per year were calculated. Rates were generated with population estimates from Vital Statistics data. Trends across pre- and post-vaccination periods were investigated through raw comparisons of these parameters by year and age group.

Results

The average rate of treated RRP per year between 2004-2013 was 0.87/100,000 (range 0.66 to 1.09) for older children and 0.91/100,000 (range 0.60 to 1.10) for younger children. The number of treatments per year in the older group averaged 1.64 (range 1.08-2.14) and in the younger group averaged 2.14 (range 1.93-2.68). No trends in treatment prevalence, age, or treatment frequency in either group were apparent over time.

Conclusion
Despite growing uptakes of HPV vaccination, the rate and frequency of treatment of RRP in NY children have not changed over time. The rarity of RRP and the delay between HPV vaccination and exposure of at-risk infants may explain these results. No central source of US RRP patients exists, however this dataset could be useful for long term monitoring which will be necessary to identify any effect of HPV vaccination on rates of RRP in children.

References


Epidemiology of cervical cancer in a region of southern Algeria

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Background / Objectives

Cervical cancer is the second most common cancer and cause of cancer-related death for women worldwide. The early stages of cervical cancer can be free of symptoms, Infection with some types of HPV is the greatest risk factor for cervical cancer. Screening is beneficial in women between 25 and 65 years old. Cytologic test is used in combination with HPV testing

Methods

It’s a prospective analysis of data about cervical cancer from 2016 to 2018 in the province (wilaya) of LAGHOUAT (Algeria). 7 towns was chosen at random. During this two-year study period, 400 cervical screening are required. Actually 200 cervical screening were made. The women age were between 25 and 64 years old.

Results

It’s was the first screening for all the 200 women, and 56 percents had only one sexual partner all their life and 12 percent had more than two

the result of cervical intraepithelial neoplasia (CIN) I, II, III was 45, 22, and 15 percent

Conclusion

in Algeria an organized screening policy must be set up to reduce the mortality rate from this cancer

References


INCREASING TRENDS IN THE INCIDENCE OF POTENTIALLY HUMAN PAPILLOMAVIRUS-ASSOCIATED HEAD NECK CANCER IN ITALY (1988-2012)

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Background / Objectives

High risk alpha human papillomaviruses (HPVs) are recognized to be causally related to a subset of head and neck squamous cell carcinoma (HNSCC) arising from the crypt epithelium of the palatine and lingual tonsils. The aim of this study was to explore the trends in the incidence of HNSCC arising from different anatomical sites potentially related and unrelated to HPV infection among Italian women and men to provide clues on possible growing impact of HPV in the epidemiology of HNSCC in Italy.

Methods

Epidemiological data were retrieved from 10 long-term Cancer Registries of the Network of Italian Cancer Registries (AIRTUM) covering a population of 7.8 million of inhabitants (13% of the whole country) in the period 1988-2012. Trends were described by means of the estimated annual percent change (APC) with appropriate 95% confidence intervals stratified by age, sex, and birth cohort and compared between HPV-related and HPV-unrelated anatomical sites. Only cases with squamous cell histology or morphologic variants of HNSCC were included in the analysis. Cancers arising from lip, nasopharynx, nasal cavity and sinuses were excluded as they are linked to other etiological factors.

Results

A total of 28,883 HNSCCs were included in the analysis. The age-standardized (on European population) annual incidence trends of all sites showed a significant decrease in males (APC: -1.61, 1988-1998; P<0.0001; APC:-3.18, 1998-2012; P<0.0001) and a significant increased in females (APC: +1.41, 1988-2012; P=0.0002). The incidence of cancers arising from head and neck sites strongly related to HPV infection (tonsil and base of tongue/lingual tonsil) increased significantly over the period 1988-2012 (APC: +1.34%; P<0.0001), particularly in females (APC: +2.59%; P=0.0029). Conversely cancers arising from sites poorly related to HPV infection decreased markedly in males and remained relatively stable in females.
Conclusion

The pattern observed suggest a potential increasing impact of HPV infection on the epidemiology of HNSCC in Italy.
CERVICAL CANCER IN SITU AMONG WOMEN AGED ABOVE 60 WHO WAS ADEQUATELY SCREENED AT 50S, AND THE POTENTIAL OF PROGRESSING TO INVASIVE CERVICAL CANCER

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Background / Objectives

Nowadays in high-income countries, many women turning age 60 have been adequately screened in their 50s. A previous study found that women who were adequately screened with no abnormality at ages 51-60 do not gain a statistically significant benefit from the extended screening test at ages 61-65 in terms of reducing subsequent invasive cervical cancer. To understand the underlying reason, we performed a population-based cohort study to investigate the cumulative incidence of cervical cancer in situ (CIS) in women aged above 60 who have been adequately screened at age 50s, aiming to predict their likely potential of progressing to invasive cervical cancer.

Methods

Women born between 1919 and 1945 who live in Sweden and had cervical screening records available since age 51 were identified in the Total Population Register. Their screening histories between ages 51-65 were retrieved from the Swedish National Cervical Screening Registry. Women who had at least two separate Pap tests at ages 51-60 without any abnormal finding were included in the analysis. CIS and invasive cervical cancer from age 61 to 80 were retrieved from the National Cancer Register. We estimated the cumulative incidence of CIS up to age 80 among women screened at ages 61-65, and compared to the cumulative incidence of invasive cervical cancer up to age 80 among women unscreened at ages 61-65.

Results

Among 332,746 women who were adequately screened in their 50s, 1.9‰ (95%CI: 1.6‰-2.2‰) of women screened at ages 61-65 were found to have CIS, and 1.6‰ (95%CI: 1.2‰-2.0‰) of women unscreened at ages 61-65 were found to have invasive cervical cancer up to age 80.

Conclusion
Women who were adequately screened in their 50s with no abnormality presented a low risk of CIS after age 60, but these precursors are very likely to progress to invasive cervical cancer since the cumulative incidence of invasive cancer among women unscreened at 61-65 is close to the cumulative incidence of CIS. Therefore, the low effectiveness of cervical screening at ages 61-65 among women being adequately screened previously may due to a low incidence of precursor lesion. However, CIS above age 60 may be risky enough to warrant careful follow-up and treatment. In the scenario that more and more women turning 60 have been adequately screened, screening all of them after age 60 with public resources may not gain satisfactory cost-benefit, given the low incidence of precursor lesions and statistically insignificant effectiveness on cancer reduction. Future studies can endeavor to identify residual risk factors for developing precursor lesions after age 60 despite being adequately screened in the past.
AGE-SPECIFIC ADDITIONAL IMPACT OF A
NOVAVALENT HPV VACCINE ON PRECANCEROUS
SQUAMOUS CERVICAL LESIONS IN SPAIN


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Background / Objectives

Nonavalent papillomavirus (HPV) vaccine has been licensed in December 2014 and is currently undergoing World Health Organization review for prequalification. Vaccination of girls with HPV bi-quadrivalent vaccines has been widely implemented in Europe and Spain. The bivalent vaccine (Cervarix®, GlaxoSmithKline) targets HPV 16/18, the quadrivalent vaccine (GARDASIL®/Silgard®, Merck&Co) target HPV 6/11/16/18 and the nonavalent vaccine (GARDASIL 9®, Merck&Co) targets HPV 6/11/16/18/31/33/45/52/58. The objective was to describe squamous precancerous cervical lesions potentially prevented by the nonavalent vaccine compared to the bi-quadrivalent vaccines according to age.

Methods

Histologically confirmed cases of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2, n=145) and grade 3-carcinoma in situ (CIN3-CIS, n=244) were studied (2009-2014) in a University Hospital in Spain. High risk HPV genotypes were detected by Linear Array HPV Genotyping test (Roche diagnostics, Mannheim, Germany). The proportion of CIN2-3 lesions potentially prevented by different vaccines was calculated for women 18 to 34, 35-44 and ≥45 years old (age group 1, 2 and 3, respectively). Cervical lesions with coinfection were attributed to the detected genotype belonging to the HPV group most commonly detected in invasive cervical cancer (hierarchical attribution). Ethics Committee of Clinical Investigation of Galicia approved this study. Women signed informed consent. Epidat 3.1 was used for statistical analysis.

Results
Bi-quadrivalent vaccines potentially prevented 59% CIN2 vs. 69% CIN3-CIS ($p<0.001$). Nonavalent vaccine potentially prevented 86% CIN2 and CIN3-CIS. Bi-quadrivalent/nonavalent vaccines potentially prevented 63/87%, 51/91% and 50/75% of CIN2 and 78/90%, 66/86% and 45/76% of CIN3-CIS in age group 1, 2 and 3, respectively. Impact of these vaccines in CIN2-3 tended to decrease with increasing age ($p$-trend $<0.05$). Potential absolute additional impact of nonavalent vaccine was 16%, 26% and 29% of CIN2-3 in age group 1, 2 and 3, respectively, ($p<0.005$).

**Conclusion**

In comparison with bi-tetravalent vaccine, nonavalent vaccine would reduce the gap between CIN2 and CIN3-CIS prevention. Although nonavalent vaccine impact on precancerous lesions decreased as women age increased, significant absolute additional impact was expected in all age groups, especially in women more than 35 years old. Age-specific impact of nonavalent vaccine should be taken into account in cost-effectiveness evaluations and in vaccinated population screening.
High human papillomavirus (HPV) concordance and transmission rates among sexually active couples have been well established. The role of HPV-genotype specific viral load concordance among couples in recently-formed relationships has not been studied well.

Methods

We used data from the prospective HITCH-cohort study. This study included young women (aged 18-24) and their male partners who had recently initiated a sexual relationship in Montreal, Canada, and the couples were followed for up to two years. In the current analyses, we analyzed their web-based questionnaires and genital samples collected at baseline and at four months. Samples were tested for HPV DNA by polymerase chain reaction (PCR) using a Linear Array HPV genotyping assay. In HPV positive samples, viral loads of HPV6, 11, 16, 18, 31, 42 and 51 were quantified using type-specific real-time PCR assays. We assessed the correlation between viral load measurements (number of HPV DNA copies per cell) by calculating Spearman’s rank-based coefficients.

Results

We analyzed 502 couples for HPV DNA, of which 233 couples had at least one partner with a genital sample positive for HPV DNA. Among men and women, 162 and 150 type-specific, persistent HPV infections were detected, respectively. Genital viral loads at baseline were correlated with viral loads at 4 months within individuals with a persistent HPV infection, to a larger extent in men (rs=0.413; p<0.001) than in women (rs=0.191, p=0.019). Furthermore, in HPV concordant couples, HPV viral loads of sexual partners were correlated with each other at baseline (142 couples, r=0.267, p=0.001). At four months, the magnitude of the viral load correlation decreased in couples that continued having sexual activity with each other (91 HPV-couples, r=0.195, p=0.064). Viral loads of men and women at baseline were not associated with type-specific viral loads of their sexual partner four months later, despite remaining sexually active (r=0.076, p=0.431; r=0.033, p=0.743, respectively).
Conclusion

In individuals with a persistent HPV infection, particularly men, one’s viral load is predictive for the viral load four months later, suggesting limited fluctuations in viral loads over time. HPV viral loads are correlated in young, recently-formed heterosexual couples, but this correlation seems to decrease as the relationship progresses.
THE ONSET OF ORAL SEX, HUMAN PAPILLOMAVIRUS 
AND OROPHARYNGEAL CANCERS 

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Background / Objectives

Human papillomavirus (HPV) is a strong risk factor for a subset of head and neck 
cancers (HNCs), primarily of the oropharynx (OPC). Sexual behaviours have been 
suggested as determinants of HPV infections in the oral cavity, but the evidence is 
inconsistent. Our objectives were to estimate the extent to which oral sex behaviour 
was associated with an increased risk of OPC, and how much of the association was 
mediated by oral HPV infection.

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 gender. Life-course oral 
sex behaviors (including age at first oral sex and time since first oral sex) was 
collected by semi-structured interviews using a life-grid technique. Oral rinse and oral 
brush specimens, collected from both cases and controls, were analyzed for alpha 
HPV genotypes by PCR protocol. Mediation models using logistic regression were 
used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the 
association between life course oral sex behaviors and risk of OPC, adjusting for
age, sex, number of educational years, lifetime number of sexual partners, lifetime smoking and alcohol drinking.

Results

A total of 188 OPC cases and 429 controls were included in the analyses. The majority were between 16 and 30 years old the first time having oral sex (63.8% and 55.2% for OPC and controls, respectively). HPV DNA was detected in 63.3% of cases and 14.2% of controls. HPV 16 genotype accounted for 76.5% and 16.4% of HPV positive cases and controls. Age at first oral sex practice was associated with OPC (adjusted OR=2.98; 95%CI 1.37-6.47). When stratified by HPV status, this association decreased (adjusted OR=1.09; 95%CI 0.25-4.71) only in the HPV-positive group. With respect to time since first oral sex, the adjusted ORs were 2.80 (95%CI 1.57-4.97) among all, and 1.04 (95%CI 0.31-3.50) in the HPV-positive group.

Conclusion

Oral sex behaviours were associated with an increased risk of OPC in Canadians, which appears to be mediated by oral HPV infection.
THE PROGNOSTIC ROLE OF DETECTION AND GENOTYPING OF HPV IN PENILE CARCINOMA

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Background / Objectives

A Penile carcinoma (PC) is a rare disease in North America and Europe, a frequent and a serious health problem in developing countries such as Brazil¹-³. More than 4600 cases of PC have been registered by the Brazilian National Cancer Institute in 2015⁴. The recurrence or persistence of the infection, including human papillomavirus (HPV), may lead or contribute to the development of penile carcinoma⁵,⁶. The prevalence of HPV changes according to the subtypes of squamous cell PC and is 30 to 50%⁷. Although it can reach almost 100% in basaloid, in situ and verrucous penile carcinoma⁸. Objective: Analyze the influence of the presence and detect the type of Human Papillomavirus (HPV) in groin metastasis and specific-cancer survival of patients with penile carcinoma, as well its association to histological variables.

Methods

This retrospective cohort study involved 113 patients with PC treated in the Uro-Oncology service of Hospital Araujo Jorge (HAJ), a unit of the Association Against Cancer in Goias, Brazil (ACCG), from January 2003 to November 2015. This study was approved by the Research Ethics Committee of HAJ. The paraffin blocks containing the cancerous tissue fragments were subjected to extraction of viral DNA using a commercial kit (Promega Corporation, USA), subsequently subjected to polymerase chain reaction testing with short PCR fragment (SPF PCR) primers to detect HPV DNA. HPV genotyping was performed using INNO-LiPA. The HPV 16 and/or 18 presence and its association to other histological profiles were evaluated in penile carcinoma (PC) patients. Uni and multivariate analysis were performed to establish the role of histopathological and HPV on the risk of inguinal metastasis and cancer-specific survival of those patients.

Results
One hundred and thirteen patients were enrolled, forty seven detected with HPV (41.5%). Almost sixty percent of the cases harbored low grade squamous cell carcinoma (SCC). The most prevalent histological subtype was the usual SCC (69.9%), followed by warty SCC (13.3%). The high histological grade (p=0.02) and the presence of HPV18 (p=0.02) were independent prognosticators for specific cancer survival (SCS). Neither the presence of HPV nor the HPV genotype were associated to a higher risk of groin metastasis.

Conclusion

The findings suggest that HPV 18 is an independent factor of poor cancer-specific survival. The overall HPV prevalence (41.5%) and genotype 16 as the most prevalent (51%), followed by HPV18 (23.4%). The importance of HPV in PC is undeniable, although multi-institutional, prospective, collaborative studies concerning this issue are still necessary to better establish its’ prevalence and prognostic impact.

References


P02-12
RECENT INCREASE IN CERVICAL CANCER INCIDENCE IN SWEDEN 2014-2015

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Background / Objectives

From 2014 a clear increase in cervical cancer incidence was observed in Sweden. Using recently available data (June 2017) from the National Cancer Register (NCR) and the National Cancer Screening Register (NKCx) the objective of this study was to investigate this increase by histology, age, and clinical stage at diagnosis.

Methods

Incidence rate ratios (IRR) with 95% confidence intervals (CI) by calendar time were estimated in multivariate Poisson regression models, with interaction terms for histology, age and clinical stage at diagnosis as mediators.

Results

There was an increase in cervical cancer incidence by around 20% (IRR=1.20, CI 1.06-1.36) from 2012 to 2015. This increase was mostly pronounced for adenocarcinoma with a 42% increase in incidence from the period 2012-2013 to 2014-2015 (IRR=1.42, CI 1.18-1.72), while for squamous, adeno-squamous and more rare types of cervical only small, statistically non-significant changes were seen in overall incidence. The increase of adenocarcinoma was mainly concentrated to FIGO stages IA and IB, with a more than 40% increase (IRR=1.45, CI 0.96-2.19 and IRR=1.48 CI 1.15-1.92, respectively). For squamous cancer there was a corresponding increase with around 20% for stage IB cancers only (IRR=1.20, CI 1.01-1.46). For adenocarcinoma the increase in incidence was more pronounced in women below age 50, and for those in stage IB cancers. For squamous cell cancer no statistically significant changes with age could be discerned for the comparison between the two time periods.

Conclusion

The main changes in incidence were seen for early clinical stages, and for adenocarcinoma for women below age 50. Whether these changes in incidence are due to an increase in the underlying risk of cervical cancer or failures of the screening organization still needs to be investigated.
Screening history and the risk of invasive cervical cancer in women aged 66 and older

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Background / Objectives

Cervical cancer is one of the most common cancers in Taiwan. In 1995, the Taiwan government was launched the cervical cancer screening program providing an annual pap smears test of women aged 30 or above. Due to this screening program, the cervical lesions could be diagnosed and treated early. The invasive cervical cancer incidence and mortality rate were decreased after screening program implemented, however, the incidence and mortality rate is still higher in elderly and higher than western countries. The aim of this study was to evaluate the association between pap smear test screening history and the risk of invasive cervical cancer at different age groups.

Methods

In this retrospective cohort study, women aged 36 years or above without cervical cancer history and alive in the end of 2009 were as study subjects. The pap smear screening history was retrospective to 2001-2009 and the incidence of cervical cancer was followed in 2010-2014. Data were obtained from the household registration database, cervical cancer screening registry database, and the cancer registry database. According to the screening history, subjects were classified into 8 groups, including A: regular screening; B: screening attendance in 2004-2006 and 2007-2009; C: screening attendance in 2004-2006, 2007-2009; D: screening attendance in 2007-2009; E: screening attendance in 2001-2004, 2004-2006; F: screening attendance in 2004-2006 sieve; G: screening attendance in 2001-2003; H: never screening. The cumulative incidence of 36-50 years, 51-65 years and over 66 years were estimated by Nelson-Aalen method and hazard ratios were estimated by Cox’s proportional hazards model

Conclusion

Our study results showed that in women over 66 years old never having pap screening test in 2001-2009 had the highest invasive cervical cancer incidence rate and hazard ratio. Women who had regular screening history, at least once in every three years, has the lowest risk of invasive cancer incidence. Moreover, the longer time interval since last pap test the greater the risk of invasive cervical cancer was increased, especially in women over 66 years. In conclusion, screening history pattern was associated with the risk of invasive cervical cancer in women aged 66 and older. And we need to further consider the feasibility and impact of whether the screening test stops at age 65 in Taiwan.
P02-14
Pilot prevalence of incidence of 12 genotype of high risk HPV and 2 genotype of low risk HPV in Khorasan Razavi State

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Background / Objectives

cervical cancer is the one of common cancer in women. humman papiloma virus is the main factor in etiology. screening of HPV and vaccination can be effective in prevention in this dieasis.

we intended to determine the prevalence of HPV and genotyping in khorasan razavi.

Methods

In order to detect antibodies against HPV in cervical spicement and DNA of HPV were extracted and tested using PCR to be indentified.

Results

Of 900 case in study, 37 cases(4/1%) werw positive for HPV, and 7 cases(18/9%)were also be positive for low risk HPV, 30 Cases(81/1%)were positive for high risk HPV. also multiple HPV determined in 7 cases. statistical analyzis by Chi-2 showed only relation between HPV and passive smoker and duration of using of pil.

Conclusion

Considering the prevalence of HPV in khorasan razavi(4/1%) is lower than report of other countries. but in all of study about efficacy of vaccination, complication and precancerous lesion decresed. we shoud spread this study in other site of iran that can be determind cost benefit of vaccination.
Presence of HPV in Inverted Papilloma

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Background / Objectives

Inverted papilloma (IP), often referred to as Schneiderian Papilloma, is a locally destructive benign tumour of the sino-nasal mucosa with a tendency for malignant transformation and a high propensity for recurrence. It arises from the transitional epithelium, the Schneiderian membrane. In a Swedish population-based study it was shown to have significantly increased over the last decades. Sinonasal SCC (squamous cell carcinoma) has been shown to be more common among patients with IP than in the general population. The etiology is unknown. Proposed etiological factors are environmental pollutants, organic solvents, smoking and chronic rhino–sinusitis. The possibility of a viral etiology has been put forward where Human Papilloma Virus (HPV) has been the most discussed. Studies on HPV and IP have shown very diverging results. The aim of this study is to analyse the presence of HPV in IP in Stockholm and to investigate if there is any correlation between presence of HPV and recurrence, dysplasia or malignant transformation in IP.

Methods

From the Swedish Cancer Registry we identified all patients diagnosed with IP in Sweden 1960-2010. From the patients in our data set diagnosed from 2001 and onwards, diagnosed in the county of Stockholm, we retrieved paraffin embedded blocks with their IP from Biobank Stockholm. After histological re-evaluation of the original diagnosis by a qualified pathologist, and loss of samples due to technical problems, 99 cases out of 126 were included in the study. By analyzing the medical reports we retrieved information about recurrence, dysplasia in the specimens and malignant transformation. The study was approved by the Ethical Committee at the Karolinska Institute, Stockholm, Sweden. The paraffin embedded tumors were cut in 2x15µ sections and DNA was extracted. Detection of HPV was done by PCR using the Magpix(Luminex). The results were confirmed with insitu hybridization.

Results
In all, 13 of the 99 specimens of IP were found to be HPV-positive. 8 were positive for HPV 11, 4 for HPV 6, and 1 for HPV 45. Among the patients with HPV, 4/13 were seen to recur and 4/13 showed dysplasia. This is to be compared with 33/86 and 8/86 respectively in patients with HPV-negative IP. None of the 13 patients with HPV-positive IP developed SCC while two of the HPV-negative did.

Conclusion

13% of the IP were HPV positive but only one with a high oncogenic risk HPV. The HPV-positive IP’s were found to have a higher rate of dysplasia compared to the non HPV-positive ( 30% vs 9%) but no difference was found in their recurrence rate. Patients with HPV-positive IP did not have a higher rate of malignant transformation.
HPV-SPECIFIC B AND T-CELL RESPONSES IN VACCINATED AND NON-VACCINATED YOUNG WOMEN.

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Background / Objectives

A primary HPV infection is cleared naturally in about 90% of the cases, suggesting that the immune system plays an important role in the protection against HPV-associated diseases, like cervical cancer. The HPV vaccines induce high HPV-specific antibody levels and memory B cell responses. Moreover, T-cells are known to be of importance in cell-mediated immunity. However, HPV-vaccine induced cell-mediated responses are not well understood after the HPV vaccination. We aim to investigate the cellular immune responses comparing non-infected, transient- and persistent infected young women.

Methods

In a longitudinal follow-up study, vaccinated and non-vaccinated young women were followed for 7 years post vaccination. From a part of all individuals peripheral blood monocytes (PBMC) were isolated (n=100 per year). Memory B-cell responses will be measured by HPV-serotype specific ELIspot assay using virus like particles, assembled from the major capsid protein L1 for HPV-16, HPV-18, HPV-31 and HPV-45. T-cell responses will be determined by cytokine production of stimulated PBMCs with HPV-specific peptides pools.

Results

Memory B-cell responses and T-cell responses of participants with persistent HPV infection will be compared with those of participants who had no HPV infection or cleared their HPV infection. These immune responses will be further evaluated in the vaccinated and non-vaccinated groups. HPV-specific frequencies of memory B-cells will be determined and T-cell responses will be expressed in numbers of HPV-specific cytokine producing T-cells.

Conclusion
Obtained results will provide more insight and understanding in the immune mechanism of HPV-specific cellular responses.
HR-HPV L1,E1,E2,E6,E7 SEROPOSITIVITY DOES NOT PREDICT ANAL HSIL AMONG HIV-POSITIVE MEN WHO HAVE SEX WITH MEN


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Background / Objectives

High-risk HPV L1 and E6/E7 seropositivity is prospectively associated with anal cancer. We studied L1, E1, E2, E6, E7 seropositivity of high-risk (hr) HPV types as potential predictors of anal high-grade squamous intraepithelial lesions (HSIL) among HIV-positive men who have sex with men (MSM).

Methods

HIV-positive participants of the longitudinal HIV&HPV in MSM (H2M) study who had at least two visits and a high-resolution anoscopy (HRA) after the last H2M visit were included in this analysis. Sera were collected in 2010-2013. Serum antibodies to E6, E7, and L1 proteins of 7 hr-HPV types (16, 18, 31, 33, 45, 52, 58), and serum antibodies to E1 and E2 of HPV16 and HPV18 were analyzed by multiplex serology. Seropositivity was defined as 3 out of 4 positive among E1/E2/E6/E7 for HPV16 and HPV18; and both E6 and E7 positive for each non-HPV16/18-type. Univariable and multivariable logistic regression was used to assess whether hr-HPV seropositivity was predictive of HSIL.

Results

Among 193 MSM (median age 50 years [IQR]:45-56) 60 (31%) were diagnosed with histologically proven anal HSIL: 25 (13%) AIN2 and 35 (18%) AIN3. The median nadir CD4+ was 235 cells/µl (IQR: 150-315 cells/µl), and 94% had an undetectable HIV viral load at time of HRA. Seropositivity for E1, E2, E6, E7 of HPV16 was 7%, 4%, 4%, and 5%, respectively. In total, 0 (0%) were HPV16 three out of four positive for E1/E2/E6/E7, and 0 (0%) HPV18 three out of four positive for E1/E2/E6/E7. E6 and E7 seropositivity for each of the non-HPV16/18 hr-HPV types was 0% (n=0).
Type-specific seropositivity as defined above was not associated with HSIL diagnoses.

Conclusion

No association between type-specific hr-HPV seropositivity and anal HSIL was found among HIV-positive MSM. Our analysis shows that (type-specific) hr-HPV seropositivity cannot be used as predictor of HSIL in HIV-positive MSM.
P04-03
SEROTYPE AND GENETIC DIVERSITY OF HUMAN PAPILLOMA VIRUS 58 IN ITALIAN WOMEN WITH LOW-GRADE CYTOLOGY

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Background / Objectives

Persistent infection with high-risk HPV genotypes is highly associated with the development of cervical cancer. HPV58 is a member of the HPV16-related alpha9 family and accounts for approximately 2% of all cervical cancer cases worldwide. Genetic variants of HPV58 have been classified into four major lineages, A, B, C and D and seven sub-lineages A1, A2, A3, B1, B2, D1 and D2, the distribution of which varies by geographical region. Lineage A predominates in all regions except in Africa, where lineages A and C are found in comparable proportions.

The aim of this study was to evaluate the potential influence of common HPV58 L1 and L2 polymorphisms on capsid protein recognition by antibodies elicited by natural infection.

Methods

HPV58 L1L2 pseudoviruses (PsVs) representing the eight major L1 and L2 variant lineages (A1, A2, A3, B1, B2, C, D1 and D2) were generated. Paired serum and DNA samples collected from women following a diagnosis of ASCUS or LSIL were tested for the presence of neutralizing antibodies against HPV58. HPV58 DNA positive samples from patients with evidence of seroconversion against HPV58 were subjected to fragment sequencing to identify their lineage variant status.

Conclusion

Among the 216 serum samples tested, 31 were seropositive against all HPV58 variants with the exception of the C variant. One serum sample was positive for HPV58 C variant, but did not recognise any other variants. Out of 32 seroconverted individuals, 21 (65%) were also DNA positive against HPV58 of which 19 were infected with HPV58 A2, one with HPV58 C, and one with HPV58 B2. We are currently mapping target specificity of these antibodies by construction of inter-lineage loop swap PsV. These data demonstrate that naturally occurring polymorphisms in the HPV58 capsid proteins affect recognition by antibodies elicited during natural infection and suggest the existence of lineage-level serotypes.
Human papillomavirus prevalence and genotype distribution in urine samples from vaccinated as compared to non-vaccinated females in Norway


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Background / Objectives

Quadrivalent HPV vaccine was included in the Norwegian childhood immunisation programme in September 2009 to girls in 7th grade. At present, 88% of all eligible girls have received at least one dose, and 86% all three vaccine doses. Since November 2016, catch up vaccination for girls up to 26 years is currently offered in a 2-year programme. In the national HPV-surveillance programme, HPV-testing in urine is used to monitor the impact of HPV vaccination on HPV prevalence and type distribution in pre-screening age. Two HPV prevalence base-line studies have previously been performed in non-vaccinated cohorts at age 17. The HPV-prevalence was 19.9% (girls born 1994) and 15.5% (girls born 1996), respectively. In this study, we include also the first vaccinated cohort (born 1997). We present preliminary results of the impact of HPV-vaccination on HPV-prevalence and genotype distribution in 17-year old girls. The study was conducted prior to the start of the catch-up programme.

Methods

Two birth cohorts of unvaccinated 17 year old girls (n=56,000) and one birth cohort of vaccinated 17 year old girls (n=30,000) were invited by mail to participate in the study. Sampling materials were sent to all girls who signed the informed consent form. The presence of HPV was investigated by using a modified GP5+/6+ PCR protocol, followed by hybridization of type-specific oligonucleotide probes coupled to fluorescence labeled polystyrene beads (Luminex suspension array technology), detecting and genotyping 37 HPV types (WHO validated protocol). Sample adequacy was evaluated through a beta-globin PCR. Individual vaccination records were retrieved from the Norwegian immunisation register, and HPV-prevalence in vaccinated and unvaccinated girls were compared.

Results

Preliminary results show a significant reduction in overall HPV prevalence in vaccinated as compared to unvaccinated 17-year old girls. The prevalence of high-risk vaccine types 16 and 18 were dramatically reduced, and also for non-vaccine
types a reduced prevalence was observed. Analyses are ongoing and detailed results will be presented in the poster.

**Conclusion**

In this large, population based study, a high effectiveness of the HPV-vaccination programme for 12 year old girls was demonstrated, and HPV-testing in urine samples was found to be easy and highly feasible for vaccine surveillance in adolescent girls. Except from HPV type 11, quadrivalent vaccine targeted HPV types (HPV 6, 11, 16 and 18) were among the most prevalent types in the unvaccinated cohorts. The prevalence of vaccine types was greatly reduced in vaccinated girls at age 17, and clear evidence of cross-protection of non-vaccine types was observed.

**References**


POTENTIAL IMPACT OF THE 9vHPV VACCINE IN SOUTH KOREA: AN OVERVIEW

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Background / Objectives

Background

In 2016, the Korean government launched a national HPV vaccination programme for 12 year old girls (bivalent and quadrivalent HPV vaccines) and approved the use of 9-valent HPV vaccine. This is expected to have a significant impact on HPV-related disease burden in Korea. The aim of this review is to examine the current burden of HPV-related cancers and disease and to estimate the relative contribution of the nine vaccine types (HPVs 16/18/31/33/45/52/58/6/11).

Methods

Methods

A comprehensive search of peer-reviewed biomedical literature was conducted to assess the burden of HPV disease in Korea by using MEDLINE, Asian Pacific Journal of Cancer Prevention, KoreaMed Synapse and Google Scholar until August 2016.

To assess the potential impact of the 9vHPV vaccine in HPV-related lesions, we used data from an international project on HPV-related lesions designed and coordinated by the Catalan Institute of Oncology (ICO) (Barcelona-Spain). Consecutive histologically confirmed paraffin-embedded cases of HPV-related anogenital cancers (cervix, vulva, vagina, anus and penis) were obtained from Korean hospital pathology archives. Cancer sites with a limited number of cases were supplemented with cases from the Asian region. HPV DNA-detection and typing was performed by using SPF10-DEIA-LiPA25 system and relative contribution was expressed as the proportion of type-specific cases among HPV positive samples.
Results

Despite a downtrend in cervical cancer rates in recent years, Korean rates still remain high in comparison to other developed countries (age-standardized rate in 2012: 9.5 cases per 100,000 women). HPV-related anogenital cancers other than cervix remain rare. Preliminary results show that the combined relative contribution of the nine HPV vaccine types was 91.3% (95% CI: 89.9-92.6) in cervical cancer, 73.6% (95% CI: 51.6-89.8) in vaginal cancer, 83.3% (95% CI: 70.7-92.1) in vulvar cancer, 88.9% (95% CI: 51.6-99.7) in penile cancer and 91.3% (95% CI: 72.0-98.9) in female anal, and 88.2% (95% CI: 63.6-98.5) in male anal cancer. The most frequently detected types in cervical cancer are HPV 16 (65%), HPV 18 (9%), HPV 33 (5%), followed by HPVs 58 (4%) and 31 (4%). HPV16 was the most frequent type in all lesions.

Conclusion

HPV-related disease burden in Korea is significant. Results suggest that the HPV types in the 9vHPV vaccine contribute to more than 90% of HPV positive female cervical and anogenital lesions. Consequently, the introduction of the 9vHPV vaccine could have a significant impact on the prevention of HPV-related cancer and disease in Korea.
SAFETY AND EFFICACY OF A QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE AGAINST PERSISTENT INFECTION AND GENITAL DISEASES IN CHINESE WOMEN DURING A 78-MONTH FOLLOW-UP

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Background / Objectives

Human Papillomavirus (HPV) infection causes significant disease burden in China. Here we report a randomized, double-blind, placebo-controlled multicenter trial conducted in Chinese healthy women to assess the safety and efficacy of a quadrivalent HPV (types 6, 11, 16, 18) L1 virus-like–particle vaccine (Gardasil®) against persistent infection and genital diseases.

Methods

3006 participants aged 20 to 45 years were enrolled and randomized (1:1) to receive HPV vaccine or placebo at Day 1, Month 2 and 6. The efficacy was followed up till Month 78. The primary efficacy endpoint was HPV 16/18-related cervical intraepithelial neoplasia grade 2 or 3 (CIN 2/3), adenocarcinoma in situ (AIS) or cervical cancer. Other efficacy endpoints included HPV 6/11/16/18-related: 1) CIN of any grade, AIS or cervical cancer (CIN plus); 2) 12-month persistent infection (PI); 3) 12-month PI, CIN plus or external genital lesions (EGLs, including genital warts, vulvar or vaginal intraepithelial neoplasia, vulvar or vaginal cancer); 4) EGLs. The efficacy analyses were done on the type-specific per-protocol efficacy (PPE) population who received all the 3 doses and were naïve to the relevant HPV types through 1 month after the third dose. Injection-site and systemic adverse events (AEs) were recorded within 15 days after each dosing. Serious AEs (SAEs) in the participants and their infants/fetuses, and pregnancy outcomes were collected throughout the study. (ClinicalTrials.gov registry: NCT00834106)

Results

0 and 7 cases of HPV 16/18-related CIN2/3, AIS or cervical cancer were observed among 1,265 and 1,237 participants in the vaccine and placebo groups, respectively, translating into an efficacy of 100% (95%CI: 32.3, 100). The efficacies against HPV 6/11/16/18-related genital diseases or infection were: 1) 100% (95%CI: 70.9, 100) for CIN plus; 2) 91.0% (95%CI: 77.7, 97.2) for 12-month PI; 3) 91.8% (95%CI: 79.8,
97.4) for 12-month PI, CIN plus or EGLs. No EGLs case was observed. 926 (61.8%) and 856 (57.1%) participants reported AEs in the vaccine and placebo groups, respectively. Injection-site AEs were more frequent in the vaccine group (37.6% vs. 27.8%, p<0.001). Systemic AEs incidences were similar (51.4% vs. 50.1%). 38 (2.5%) and 43 (2.9%) participants reported SAEs in the vaccine and placebo groups, respectively. Incidences of congenital anomaly in infants and aborted fetuses were 2.3% (11/488) in vaccine group and 1.4% (6/444) in placebo group (p=0.3371).

Conclusion

The quadrivalent HPV vaccine demonstrated good safety profile and high efficacy against persistent infection, any-grade and high-grade cervical precancerous lesions in Chinese healthy adult women.
POTENTIAL OF HPV VACCINATION IN CANCER CONTROL

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Background / Objectives

Human papillomavirus (HPV) is involved in the pathogenesis of anogenital cancers and oropharyngeal cancer (OPC) in both women and men. HPV-vaccination is approved for prophylactic use in both genders. HPV-related cancer is increasing in men, but in most countries, including Denmark, HPV-vaccination is recommended for girls only. The objective of this study was to estimate the burden of cancers caused by HPV in women and men and the potential of HPV-vaccination in cancer control.

Methods

We retrieved data on the prevalence of HPV and the genotype distribution in HPV-related cancer types from the existing literature. Data on cancer incidences as well as frequency of procedures related to cervical screening was searched from the NORDCAN database and Danish National Health Registers.

Results

Every year, 376 Danish women are diagnosed with cervical cancer and 99 women die from the disease. The incidence of cervical cancer has declined from 44.4 per 100,000 before cervical screening was introduced in Denmark in the 1960’s to 14.5 per 100,000 today. In Denmark, 700 women and 384 men are diagnosed with an HPV-related cancer each year. The annual burden of HPV-caused cancer was estimated to 548 new cases in women compared to 234 cases in men. The Danish cervical screening program is estimated to prevent 800-1300 cervical cancer cases each year. If these preventable cases are included in the estimate, the burden of HPV-caused cancer is 6-8 times higher in women than in men.

Conclusion

The burden of cancer caused by HPV and the preventive potential of HPV-vaccination is twice as high in women compared to men. However, what we see today is only the tip of the iceberg. When the cervical cancer cases prevented by screening are taken into account, the burden of HPV-caused cancer and the preventive potential of HPV-vaccination is considerably higher in women than in men. In Denmark, the coverage of HPV-vaccination has declined dramatically due to public concern about possible side-effects. Gender-neutral HPV-vaccination potentially would benefit girls more than boys, and the coverage in boys would have to be very high if coverage in girls is low.
HPV vaccination and risk of chronic fatigue syndrome/myalgic encephalomyelitis: A nationwide register-based study from Norway


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Background / Objectives

Vaccination has been suggested in the aetiology of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). However, for the vaccines studied, including the bivalent HPV vaccine, no associations have been found [1, 6-9]. The risk of developing CFS/ME after vaccination with the quadrivalent HPV-vaccine has not been studied. Recently, an increased risk of two syndromes with symptoms that partly overlap with CFS/ME (postural orthostatic tachycardia syndrome and complex regional pain syndrome) after HPV vaccination has been suspected [10-12]. From 2009, quadrivalent HPV vaccine has been offered to 12 year old girls in the Norwegian Childhood Immunisation Programme. We studied whether HPV vaccination was associated with risk of CFS/ME and assessed medical history in relation to both risk of CFS/ME and HPV vaccine uptake. Uptake of at least one dose increased from 70% to 88 % in the study period, 2009-2014.

Methods

We linked individual data from national registries, including the population registry, the patient registry and the immunisation registry using the unique personal identification number.

Yearly incidence rates of CFS/ME for 2009–2014 were calculated among all boys and girls, aged 10–17 living in Norway during the period, n=824,133.

Girls born 1997–2002 were eligible for HPV vaccination and included in analyses of the interplay between vaccination, medical history and CFS/ME, n=176,453.

We calculated hazard ratios (HRs) of CFS/ME using Cox regression. Risk differences (RDs) of vaccine uptake were calculated with binomial regression.

Results
Although the incidence of CFS/ME was higher among girls than boys, we observed a similar yearly increase in incidence rate of CFS/ME among girls and boys.

Among girls eligible for HPV vaccination, the risk of CFS/ME increased with increasing number of previous hospital visits, HR=5.23 (95% CI 3.66–7.49) for seven or more visits as compared to no visits. Having seven or more hospital visits was associated with a lower HPV vaccine uptake, RD=−5.5% (95% CI -6.7%—4.2%).

We observed no association between HPV vaccination and risk of CFS/ME, HR=0.86 (95% CI 0.69–1.08) for the entire follow-up period and 0.96 (95% CI 0.64–1.43) for the first two years after vaccination.

Conclusion

We observed an increase in CFS/ME incidence during 2009–2014. The increase was similar in girls and boys.

Further, an association between medical history and risk of CFS/ME was observed, and the risk increased with increasing number of hospital visits.

Analysing individual data, no indication of increased risk of CFS/ME following HPV vaccination was found among 176,453 girls offered HPV vaccine through the national immunisation programme in Norway 2009-2014.

References


vaccine: another angle of the 'autoimmune (auto-inflammatory) syndrome induced by adjuvants' (ASIA). Immunologic research. 2014;60:376-83.


HPV vaccination of adolescent girls is not associated with sexual activity initiation and risky sexual behaviours

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Background / Objectives

Some fear that HPV vaccination may lead to an increase in unfavourable sexual health outcomes, based on the theory of risk compensation suggesting that the relative assurance of the protection from vaccination could be associated with an increase in risky sexual behaviours. Our study aimed to test whether receiving an additional dose of Q-HPV vaccine between the ages of 13 and 15, five years after the initial dose was received, would lead to more sexual activity and more risky sexual activity over a year, among teenage girls vaccinated in Quebec, Canada.

Methods

We analyzed data collected as part of an ongoing randomized trial, ICI-VPH, investigating the role of a booster dose of HPV vaccine. All participants received 2 doses of Q-HPV vaccine in fourth grade. The intervention group received an additional dose 60 months after their first one; the control group did not receive a vaccine booster dose. Girls included in the present analysis were those who had no sexual experience at the time of the randomization and who responded to the follow-up questionnaire one year later. The main outcome was the occurrence of the first sexual experience in the year following randomization. Secondary outcomes included: lifetime number of sexual partners, condom use, STIs and pregnancy.

Results

Of 1581 girls, 798 (50.5%) received an additional Q-HPV vaccine dose and 783 (49.5%) did not. At the time of randomization, groups showed similar characteristics: the mean age was 14.8 years, 70.5% self-identified as French Canadian only, 91.3% were born in Canada, 12.0% were using hormonal contraception and 4.5% were smokers. In the year following randomization, similar proportions of participants initiated sexual activity (17.2% vs 19.9%; p-value 0.26); initiated intercourse (14.9% vs 16.4%; p-value 0.24); and used condoms (67.5% versus 63.4%; p-value 0.57). Only 2 participants reported an STI (one in each study group), and one reported a pregnancy (in the control group). In multivariate analysis, identifying as French Canadian only (OR 1.5; 95% CI: 1.1-2.0), tobacco smoking (OR 3.0; 95% CI: 1.8-5.1) and hormonal contraception use (OR 2.4; 95% CI: 1.7-3.4) were associated with sexual activity initiation.
Conclusion

We did not observe an increase in sexual activity, risky sexual behaviours or unfavourable sexual health outcomes in adolescent girls who received an additional dose of HPV vaccine between 13 and 15 years of age.
CROSS-PROTECTIVE EFFECTIVENESS OF AS04-HPV-16/18 VACCINATION IN REDUCING CERVICAL HPV INFECTIONS IN ADOLESCENT GIRLS – RESULTS FROM A COMMUNITY-RANDOMIZED TRIAL

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Background / Objectives

Aside of the high protection against the most prevalent carcinogenic HPV types (16/18) provided by the AS04-adjuvanted HPV-16/18 vaccine (AS04-HPV-16/18v), large efficacy trials have evidenced its protective effect against some non-vaccine oncogenic types. We present results from a post-hoc analysis on cross-protective vaccine effectiveness (VE) against non-vaccine HPV type cervical infections in adolescent girls from a large phase III/IV, community-randomized, controlled study (NCT00534638).

Methods

From 2007 to 2010, 22,444 girls and 11,968 boys from Finland born 1992-95 (aged 12-15 years) were allocated to 3 arms. Around ninety percent of vaccinated girls and boys in arm A (8,235/9,203) and of vaccinated girls in arm B (6,601/7,367) received AS04-HPV-16/18v. Other vaccinated subjects in arms A and B (6,614) and all in arm C (10,724) received hepatitis B virus vaccine.

HPV DNA prevalence of 14 high-risk and 11 low-risk types in cervical samples collected from female subjects when they were 18.5-19 years old was determined by SPF-10 line probe assay (LiPA) and Multiplex Type-specific PCR.

VE was calculated as a relative reduction of HPV prevalence by type in cervical samples among HPV-vaccinated girls from pooled arms A & B compared with non HPV-vaccinated girls from arm C (control arm). The analysis was performed on the total enrolled cohort (TEC), overall and by birth cohort (92-93 and 94-95), accounting for the differences in average age at vaccination (14-15 and 13-14 years old) and time to follow-up (3-4 and 5-6 years).

Results

VE are presented for HPV-31, 33, 35 and 45 (no significant changes were shown for other types).
<table>
<thead>
<tr>
<th>HPV type</th>
<th>TEC</th>
<th>Arm</th>
<th>N</th>
<th>n</th>
<th>VE (%)</th>
<th>95% CI (LL-UL)</th>
<th>p-value</th>
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<td></td>
<td>A&amp;B</td>
<td>5,853</td>
<td>51</td>
<td>81.1</td>
<td>70.7–87.8</td>
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<td>31</td>
<td>Overall</td>
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<td>140</td>
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<td>92-93</td>
<td>A&amp;B</td>
<td>3,075</td>
<td>26</td>
<td>79.3</td>
<td>62.1–88.7</td>
<td>&lt;0.001</td>
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<td>2,778</td>
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<td>80.3</td>
<td>68.1–87.8</td>
<td>&lt;0.001</td>
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<td>Overall</td>
<td>A&amp;B</td>
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<td>121</td>
<td>47.4</td>
<td>29.2–60.9</td>
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<td>92-93</td>
<td>A&amp;B</td>
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<td>A&amp;B</td>
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<td>35</td>
<td>Overall</td>
<td>A&amp;B</td>
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<td>43</td>
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CI: confidence interval; LL-UL: lower and upper limits; N: number of subjects; n: number of positive samples; TEC: total enrolled cohort; VE: vaccine effectiveness

All analyses are exploratory. CI and p-value are based on Mantel-Haenszel adjusted for clustering and stratified by the historical HPV-16/18 seroprevalence used in the randomization.

Conclusion

Cross-protective effectiveness of AS04-HPV-16/18v against non-vaccine HPV type (31/33/35/45) cervical infections was observed in adolescent girls 3-6 years post vaccination. Protection appeared higher in younger birth cohorts.

References
Funding: GlaxoSmithKline Biologicals SA

*On behalf of the HPV-040 study group
EFFICACY OF THE QUADRIVALENT HPV VACCINE IN CERVICAL CANCER PREVENTION STRATEGY IN THE GAMBIA.

Persistent infection with high risk Human Papillomavirus (HR HPV) genotype causes 80% of cervical cancers. HR HPV 16 and 18 are responsible for 70% of cervical cancers, worldwide. Three prophylactic HPV vaccines have been developed to prevent HPV infections. In the Gambia, cervical cancer is the most frequent diagnosed cancer representing approximately 30% of all female cancers. The quadrivalent HPV vaccine, which targets genotypes 16, 18, 6 and 11 was recently piloted in the West Coast Region where majority of cervical cancer cases were reported. In order to evaluate the potential efficacy of the quadrivalent vaccine, this study assessed regional genotype distribution to ensure the HPV vaccine prevention strategy would be effective.

Methods

232 endocervical samples were collected from women age 20 - 49 years old residing in Banjul and West Coast Region. DNA was isolated using the QIAamp DNA Mini Kit (Qiagen). HPV detection was carried out by PCR amplification using primer sets PGMY09/11, which target the (L1) Major capsid gene of the virus. Genotyping was performed by Sanger sequencing technique.

Results

Eight different HR HPV genotypes were identified. HPV 52 (28.6%) was the most prevalent genotype, followed by 58 and 51 (both 14.2%). HPV 16 (7.1%) was the seventh most common genotype identified and HPV 18 was not detected. HR HPV distribution was higher in the 26-30 age group. HPV 61 was the most common low risk genotype isolated. Sequence analysis showed all HR genotypes detected were not homologous to African isolates but isolates originated from America, Europe and Asia.

Conclusion
The success of a cervical cancer vaccine prevention strategy should consider the dominant circulating HR HPV type. In the Gambia, the vaccine currently available may be of limited use.
MOTHER TO INFANT TRANSFER OF ANTI HPV 6 AND 11 ANTIBODIES UPON IMMUNIZATION WITH THE 9VHPV VACCINE

A. Joshi, A. Luxembourg

Merck & Co., Inc., Kenilworth, NJ (United States of America)

Background / Objectives

HPV types 6/11 can cause recurrent respiratory papillomatosis (RRP), a rare disease with likely mother to child transmission. This exploratory analysis was conducted to characterize the level of HPV types 6/11 antibodies in peripartum maternal blood and in cord blood of infants born to women who received 9-valent HPV (types 6/11/16/18/31/33/45/52/58) (9vHPV) vaccine in the pivotal efficacy study of the 9vHPV vaccine (V503-001, NCT 00543543). Immunization with the 9vHPV vaccine has been shown to elicit marked antibody responses to all 9 vaccine types; however limited data exist on the maternal transfer of anti HPV antibodies.

Methods

The overall efficacy study enrolled over 14,000 subjects who were randomized to 9vHPV vaccine or quadrivalent HPV (qHPV) vaccine. Participation in the sub study to assess mother to infant HPV antibody transfer was voluntary. The analysis included all mothers and infants for whom valid results for maternal blood and infant cord blood samples were available at the time of delivery. A total of 20 mother-infant pairs for HPV 6 (n=9; 9vHPV group and n=11; qHPV group) and 21 mother-infant pairs for HPV 11 (n=9; 9vHPV group; n=12; qHPV group) were analyzed. Geometric mean titers (GMTs) and seropositivity rates of anti-HPV 6/11 neutralizing antibodies in the mother-infant pair samples were assessed using competitive Luminex immunoassay.

Results

All mothers and all infants were seropositive for HPV 6 and HPV 11. Anti-HPV 6/11 GMTs in peripartum maternal blood and infant cord blood were highly correlated. The GMT ratios of peripartum maternal blood vs. those in cord blood were 1.23 (95% C.I.; 0.43, 3.49) for HPV 6 and 1.29 (95% C.I.; 0.54, 3.07) for HPV 11 in the 9v HPV group and 1.33 (95% C.I.; 0.41, 4.29 for HPV 6 and 1.19 (95% C.I.; 0.45, 3.13) for HPV 11 in the qHPV group, respectively.

Conclusion

These results indicate that antibodies induced by the 9vHPV vaccine cross the placenta and could potentially protect newborns against acquisition of vaccine type HPV related disease, such as RRP. These results mirror similar observations previously made with qHPV vaccine.
P05-09
Long Term Immunogenicity, Efficacy and Safety of 9-valent HPV vaccine in Preadolescents and Adolescents.

S.E. Olsson 1, A. Luxembourg 2

1Karolinska Institute at Danderyd Hospital, Stockholm, Sweden (Sweden),
2Merck & Co., Inc., Kenilworth, NJ, USA (United States of America)

Background / Objectives

The efficacy of the 9vHPV vaccine, developed to prevent HPV infection and disease caused by HPV6/11/16/18/31/33/45/52/58, was demonstrated in a Phase III study (Study 001) in young women (aged 16 to 26 years). In another Phase III study (Study 002), the efficacy results were bridged to girls and boys (aged 9-15 years) based on the demonstration of non-inferior HPV antibody responses compared to young women. Study 002 was extended to evaluate vaccine immunogenicity, efficacy and safety over 10 years. An interim analysis of immunogenicity of Study 002 up to 3 years and plans for longer term immunogenicity and effectiveness follow up will be presented.

Methods

Young women aged 16-26 years (Study 001) and girls and boys aged 9-15 years (Study 002) received 3 doses of 9vHPV vaccine at day1, month 2 and month 6. Serology was assessed at month 7, 12, 24, 36, using HPV-9 cLIA. Vaccine immunogenicity is estimated in the per-protocol population by assessing geometric mean titers (GMTs) and seropositivity rates to each vaccine type HPV. Non-overlapping 95% confidence intervals were used as indicators of differences of immune response.

Results

In Study 002, seropositivity rates to each of the 9 HPV types in girls and boys ranged from 99.9% to 100% at month 7 and from 93.8% to 99.7% at month 36. GMTs peaked at month 7, and decreased thereafter to plateau between month 24 and month 36. An analysis by age strata (9-12 years and 13-15 years at enrollment) showed that the month 36 seropositivity rates ranged from 96.5% to 99.7% in the younger group and 87.2% to 99.7% in the older group. This difference in GMTs by age strata was statistically significant in girls at all-time points; differences in boys were smaller and were not statistically significant.
Efficacy of the 9vHPV vaccine was established through 6 years of follow-up (median 4 years) in young women in Study 001. A cross-study comparison showed that GMTs in girls and boys from Study 002 were higher than GMTs in young women from Study 001 at month 7, and remained higher throughout the study. Based on these results, efficacy in girls and boys through month 36 is inferred. Study 002 was extended to continue assessment of antibody persistence and initiate assessment of effectiveness (through 10 years post vaccination).

Conclusion

Administration of the 9vHPV vaccine in girls and boys aged 9-15 years resulted in HPV antibody responses that persisted through 3 years. HPV antibody responses remained higher in girls and boys than in young women (the population used to establish 9vHPV vaccine efficacy) for this entire study period. Longer term assessment of immunogenicity and effectiveness is ongoing.
Would the Restoration of the Vaginal Microbiota Help the HPV Regression?

L. Serrano 1, J. Cortés 2, A.C. López 3, S. González 1, S. Palacios 4, D. Dexeus 5, C. Centeno 6

1Centro Médico Gabinete Velázquez (Madrid) (Spain), 2Private Practice (Palma de Mallorca) (Spain), 3Hospital Quironsalud (Málaga) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women’s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain)

Background / Objectives

There is increased evidence of higher diversity of the vaginal microbiota of HPV-positive Vs HPV-negative women. Bacterial species among HPV infected patients are possible cytokine profile modifying agents (Th1 to Th2), causing local immunosuppression resulting in HPV persistency. Thus, re-balance or normalization of the microbiota, may help to produce a more hostile microenvironment for HPV, thereby making easier its clearance

Methods

Review of 3 prospective studies:

- Exploratory, non-comparative, prospective, real life study conducted on healthy women aged 18 - 45 years, once daily application of Papilocare® for 12 consecutive days to measure changes in vaginal microbiota

- Prospective, non-controlled observational study including 21 sexually active positive-HPV women aged > 25y with negative pap and no colposcopy cervical lesions. Papilocare® once daily for 21 consecutive days to evaluate changes in vaginal microbiota by pyrosequencing

- Randomized, open, parallel group, controlled clinical trial to evaluate the efficacy of Papilocare® to both normalize cytology and clear HPV, in HPV-positive women with ASCUS or LSIL alterations and consistent colposcopy image

Results

- First study showed a trend of improvement (21.2% final vs baseline) of vaginal microbiota

- Second study showed a significant improvement in the cervix mucosa epithelialization vs baseline. Evaluation of changes in vaginal microbiota by pyrosequencing are under analysis and will be disclosed during Congress

Third study interim analysis:
- At 3 months, 69.2% of patients using Papilocare® (n=26) negativized pap and
colposcopy vs. 33.3% in control group (n=15) (p=0.048; Fisher test). This difference is even more evident in high risk genotype population: 67% vs 20% for PapilocareR (n=18) and control group (n=10), respectively (p=0.046; Fisher test).

-At 6 months, a positive trend of Papilocare® vs control in normalizing Pap and colposcopy in high risk genotype population: 73% vs 40% in PapilocareR (n=11) and control groups (n=5), respectively (p=ns)

-At 6 months, a positive trend to clear HPV in Papilocare® vs control group: 56% vs 30% of patients cleared HPV, respectively (p=ns). This positive trend was even more evident in high risk genotype population: 50% of patients in PapilocareR group (n=12) showed HPV cleared vs 17% (n=6) in control group (p=ns)

**Conclusion**

Papilocare® shows a postive outcome on vaginal microbiota which may enhance local immunity and might explain that Papilocare® shows a significant normalization Pap at 3 months vs control and a positive trend in both HPV clearance at 6 months with higher differences in high risk HPV patients. These findings need to be confirmed upon study completion.
P07-01
EFFICACY OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL TO REPAIR CERVICAL MUCOSA WITH HPV LESIONS. INTERIM ANALYSIS RESULTS

L. Serrano 1, J. Cortés 2, A.C. López 3, S. González 1, S. Palacios 4, D. Dexeus 5, C. Centeno 6

1Centro Médico Gabinete Velázquez (Madrid) (Spain), 2Private Practice (Palma de Mallorca) (Spain), 3Hospital Quironsalud (Málaga) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women´s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain)

Background / Objectives

To evaluate the efficacy of Papilocare® -a Coriolus versicolor-based vaginal gel- to repair cervical mucosa in women with HPV-related pap alterations and consistent colposcopy image.

Methods

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US or LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment. Preliminary analysis of percentage of patients with normal pap and concordant colposcopy image at 3 months in both total and high risk genotype virus population are presented. Citologies evaluation has been centrally-conducted in IECM laboratory (Lugo, Spain). Papilocare® arms (A+B) were combined for this analysis.

Results

Data from 41 patients at 3 months are available. 69.2% of patients using Papilocare® (n=26) had negative pap and colposcopy vs. 33.3% in control group (n=15) (p=0.048; Fisher test).

High risk genotypes virus were detected in 28 patients. At 3 months, normal pap and concordant colposcopy image was observed in 67% of patients using Papilocare® (n=18) vs 20% of patients in control group (n=10) (p=0.046; Fisher test).

Conclusion

In these preliminary results, Papilocare® shows a significant difference in repairing HPV-cervical lesions at 3 months versus control; these findings need to be confirmed upon study completion.
EFFICACY OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL TO CLEAR HPV. INTERIM ANALYSIS RESULTS

J. Cortés 1, A.C. López 2, S. González 2, L. Serrano 3, S. Palacios 4, D. Dexeus 5, C. Centeno 6, Y. Gaslain 7

1Private Practice (Palma de Mallorca) (Spain), 2Hospital Quironsalud (Málaga) (Spain), 3Centro Médico Gabinete Velázquez (Madrid) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women’s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain), 7Procare Health SL (Barcelona) (Spain)

Background / Objectives

To evaluate the efficacy of Papilocare® -a Coriolus versicolor-based vaginal gel- to clear HPV at 6 months.

Methods

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US or LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment. Preliminary analysis of percentage of patients with HPV clearance at 6 months in both total and high risk genotype virus population are presented. HPV genomic evaluation has been centrally-conducted in IECM laboratory (Lugo, Spain). Papilocare® arms (A+B) were combined for this analysis.

Results

Data about HPV clearance from 26 patients are available. HPV clearance was observed in 56% of patients using Papilocare® (n=16) vs 30% in control group (n=10) (p=0.247; Fisher test).

High risk genotypes viruses were detected in 18 patients. At 6 months, 50% of patients in Papilocare® group (n=12) showed HPV clearance vs 17% (n=6) in control group (p=0.315; Fisher test).

Conclusion

In these preliminary results, Papilocare® shows a positive trend in HPV clearance at 6 months, especially in high risk genotype virus population; these findings need to be confirmed upon study completion.
USE OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL IN PATIENTS WITH PRECANCEROUS HPV LESIONS. INTERIM ANALYSIS RESULTS


1Private Practice (Palma de Mallorca) (Spain), 2Hospital Quironsalud (Málaga) (Spain), 3Centro Médico Gabinete Velázquez (Madrid) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women´s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain), 7Procare Health SL (Barcelona) (Spain)

**Background / Objectives**

To evaluate the efficacy of Papilocare®-a Coriolus versicolor-based vaginal gel- to repair cervical mucosa in women with HPV-related cytology alterations and consistent colposcopy image.

**Methods**

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US, LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment as usual practice. Interim analysis of secondary endpoints - changes in epithelialization of the cervix evaluated by standard colposcopy and in perceived stress evaluated by PSS14- are presented. Papilocare® arms (A+B) were combined for this evaluation.

**Results**

Data from 47 patients at 3 months and 29 patients at 6 months are available. 20.7% and 47.5% of patients in Papilocare® group improved the cervix epithelialization at month 3 and 6 respectively vs 22.2% and 16.7 in control group (p=ns). A trend to stress reduction vs basal was observed in the treatment group at month 3 (-0.9 points) and was significant at month 6 (-2.9; p=0.045, Student’s t-test). Patients in control group showed a trend to stress increase at month 3 and 6 (+0.5 and +4.7; p=ns). There were not significant differences between treatment groups.

**Conclusion**

In these interim analysis results, Papilocare® shows a positive trend in cervix epithelialization and a significant stress reduction; these findings need to be confirmed upon study completion.
EFFECT OF A NON-HORMONAL CORIOLUS VERSICOLOR VAGINAL GEL AMONG POSITIVE-HPV WOMEN WITH NO COLPOSCOPY CERVICAL LESIONS. A PILOT STUDY.

S. González, L. Serrano, C. Emsellem

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Background / Objectives

To evaluate the effect of a Coriolus versicolor-based vaginal gel (Papilocare®) on cervical epithelialization in positive-HPV women with no colposcopy lesions.

Methods

An exploratory, prospective, observational study. Sexually active positive-HPV women aged > 25y with negative pap and no colposcopy cervical lesions were included during routine clinical visits and treated with Papilocare® once daily for 21 consecutive days. Primary endpoint: change vs baseline in epithelialization degree of the cervix mucosa evaluated by standard colposcopy and rated by investigator from 5 = No ectopy o 1= severe ectopy and bleeding. Secondary endpoints: 1) changes in vaginal signs and symptoms evaluated by likert-type scale from 7= severity to 28= absence, 2) changes in vaginal microbiota evaluated by pyrosequencing and 3) patient satisfaction.

Results

21 patients were included. Papilocare® showed a positive trend to improve the re-epithelialization of the cervix: mean score improved 20% (3.79 vs 4.47 baseline vs final; T test p<0.006). 52.6% of patients improved cervix epithelialization and a score of 5 was observed in 63% of women. A trend to improve symptoms was observed despite of few symptoms at baseline: 71% of patients reached maximum symptoms score at the end of treatment period. Eight patients improved the symptoms score and 3 worsened. A “moderate/complete satisfaction” and some degree of «positive impact on wellness» were reported by 84% and 90% of evaluated patients, respectively. Vaginal microbiota analysis is currently ongoing.

Conclusion

In this pilot study, Papilocare® shows promising benefits in the variables analyzed; these findings need to be confirmed in a larger study.
Background / Objectives

Multiplex Probe Amplification (MPA) is a patented real-time PCR-based technology allowing detection and genotyping of up to 20 different targets in a single closed-tube reaction, thus significantly increases throughput capability. Papilloplex™ HR HPV is a CE IVD marked product for qualitative detection and differentiation of all 14 high-risk HPV types in a single analysis.

In the present study, we carried out a comparative analysis of the performance of Papilloplex™ HR HPV test with other well established assays on clinical samples. Analytical specificity of the assay was also interrogated using the WHO HPV LabNet proficiency panel.

Methods

The Papilloplex™ HR HPV test was applied to 500 disease enriched cervical liquid based cytology samples obtained from the Scottish HPV Archive, Edinburgh, with known concurrent pathology results. Samples were also tested using the Abbott RealTime High Risk HPV assay, the Qiagen digene HC2 HPV DNA Test, the Diamex Optiplex HPV Genotyping kit and Roche Linear Array® HPV Genotyping Test. Concordance between the comparator assays and Papilloplex™ HR HPV was performed using both binomial and McNemar’s test.

Results

The Papilloplex™ HR HPV was able to detect and genotype high risk 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 up to 5 genome copy numbers for HPV 16 and 18 in WHO LabNet samples with 100% accuracy for genotyping. No significant difference in the qualitative detection of high risk HPV was observed between the Papilloplex™ HR HPV and the four assays described above. Type-specific concordance was also high.

Conclusion
In conclusion, this study shows that the Papilloplex™ HR HPV is efficient in combined screening and genotyping of HPV DNA. The current commercially available probe-based methods are limited to detection of only one target sequence per fluorescence channel. The MPA technology overcomes this limitation, allowing 14 targets to be detected and quantified in a single closed-tube reaction.

These data indicate that the analytical performance of Papilloplex™ HR HPV is comparable to established HPV assays at the level of generic high risk HPV detection and at the type-specific level. The assay shows potential promise from both disease management and epidemiological perspectives.
Development and validation of HPV test intended for use in cervical cancer screening “AmpliSens HPV HCR screen-titr-14-FL”

M. Dmitryukova, O. Kuleshova, O. Shipulina

Central Research Institute for Epidemiology (Russian federation)

Background / Objectives

By now, molecular diagnostics of HPV is not implemented in cervical cancer screening program in Russian Federation. Nonetheless, HPV testing becomes more and more popular among clinicians, gynecologists and oncologists. Thus, need for modern screening test consistent with new scientific data and acceptable under restricted conditions has emerged.

Methods

“AmpliSens HPV HCR screen-titr–14-FL” assay allows quantifying of 14 most oncogenic HPV types with simultaneous typing of 16, 18, and 45 types in one tube. By amplifying two HPV genome region (E1/E6) the assay can distinguish non-integrated and fully integrated forms of 16, 18 and 45 types.

For clinical validation 900 samples were tested in compare to HC2 test (Qiagen). Among them 100 specimens diagnosed HSIL/CC and 800 – NILM/LSIL. Besides them, 6246 samples were tested to establish clinically meaningful cutoff based on HPV viral load. The samples were obtained during screening testing in commercial laboratories and oncological centers of Russian Federation.

Conclusion

Considering samples tested against comparator test there was 97.7% overall agreement, an 96.4% positive agreement, kappa value 0.94 (95% CI 0.92 – 0.96). Negative cytology specimens showed 97.8% overall agreement, 93.3% positive agreement, kappa value 0.93 (95% CI 0.89 – 0.96). For abnormal cytology (only 45 specimens) there was 97.7% overall agreement, 100% positive agreement, kappa value 0.95 (95% CI 0.87 – 1).

Considering clinical samples, specimens with HSIL/CC diagnose have mean viral load 5.9lg (CI 95% 4.8 – 7.0). In 228 NILM/LSIL samples mean viral load was 4.5 lg (CI 95% 2.1-6.9 lg). The cutoff was established by 3.0 lg per 10^5 epithelial cells with clinical sensitivity 98.6% (CI 95% 88.8 – 98.6) and clinical specificity 87.5% (CI 95% 85.2-89.9).
MASS SPECTROMETRY AS A RELIABLE HIGH THROUGHPUT TECHNOLOGY FOR ROUTINE HPV DIAGNOSTICS

P. Wandernoth 1, M. Kriegsmann 2, C. Groh Mohanu 1, N. Arens 1, J. Kriegsmann 3

1Institute of Molecular Pathology, Trier (Germany), 2Institute of Pathology, University of Heidelberg (Germany), 3MVZ for Histology, Cytology and Molecular Diagnostics, Trier (Germany)

Background / Objectives

About 90% of cervical cancer is caused by the infection with special subtypes of Human Papilloma Virus (HPV).1 High-risk HPV tests were recently approved by the U.S. Food and Drug Administration (FDA) as a primary screening tool for cervical cancer risk in woman aged 25-65 years without a simultaneous Pap smear. Molecular test systems are required to detect high- but also low-risk HPV subtypes with high specificity and sensitivity in a time and cost effective manner. MALDI-TOF Mass Spectrometry System (MassARRAY®, Agena Bioscience, Inc.) has the potential to meet these requirements.

Methods

The HPV MassARRAY® panel detects 19 specific oncogenic HPV genotypes in one single multiplex reaction. We analyzed 10 liquid based cytology samples and compared the results with the RT-PCR based COBAS and the hybridization based HPV LCD-array system.

Results

All high risk HPV subtypes detected by the COBAS system or the HPV LCD-array were also identified by MassARRAY®. Whereas the COBAS system detected a maximal number of 3 HPV types (16, 18 and twelve other genotypes without subtyping), the MassARRAY® and the HPV LCD-array could discern further HPV subtypes in several patients.

Conclusion

We conclude that the MassARRAY® HPV assay represents a highly specific, sensitive, reliable and cost-efficient method for the detection of HPV subtypes in liquid samples (and FFPE samples 2) in a high throughput setting.

References

Background / Objectives

HPV infection is the known cause of cervical cancer and in Sweden, the recommendation for primary HPV testing in cervical cancer screening was initiated in 2016. In the Örebro region, the Aptima (Hologic) HPV assay is used for primary HPV testing, detecting mRNA from 14 hrHPV genotypes, however without distinction between types and also without a human control gene for verification of sample adequacy. The Aptima assay is an in vitro amplification test for qualitative detection of E6/E7 viral messenger RNA (mRNA) but the expression level in a sample might not always correlate with the magnitude of a positive assay signal, especially for samples near the assays detection limit. Using the sensitive digital droplet PCR method we aim to compare mRNA and DNA levels in clinical samples as well as establishing laboratory cut-off levels that can be used as internal controls.

Methods

This ongoing study includes hrHPV positive samples from the primary HPV screening in Örebro, Sweden. Analyzed Aptima sample tubes are collected and 200 µl of residual sample extracted for sample DNA. Genotyping using extracted DNA is performed with Anyplex II HPV28 (Seegene). For HPV16, -18, 33 and 45 positive samples, corresponding liquid based ThinPrep cytology (LBC) vials are retrieved and used for both RNA and DNA extraction. Digital droplet PCR is performed in parallel for both DNA and mRNA amplicons using primer and probesets for E6/E7 of genotypes 16, 18, 33 and 45, also including the human control gene HBB.

Results

Results will be presented at congress.

Conclusion

Results will be presented at congress.
Population-based HPV Testing Performance: Comparison HC2 and Cervista HPV Testing Assays

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Background / Objectives

Human papillomavirus (HPV) and Pap cytology co-testing has been used in our institution for cervical cancer screening and post therapy surveillance. In our patient population, HPV testing performance by Hybrid Capture 2 (HC2, Qiagen, Valencia, CA) or Cervista HPV assay (Hologic Inc., Bedford, MA) is limited.

Methods

We retrospectively searched our institution’s database for women aged 30 years or 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). HC2 HPV assay (2007-2010) or Cervista HPV assays (2011-2016) were used in both clinics. A total of 16,214 women from CPC (mean age, 55 years; 30-91 years) and 10,588 women from GYN clinics (mean age, 51 years; 30-96 years) were included in the study. HC2 and Cervista HPV assays were compared by HPV testing results stratified by Pap test results in both clinics. The differences were analyzed by Fisher’s exact test. A total of 175 follow-up biopsies from women who visited GYN clinic and had HSIL/carcinoma Pap results were reviewed. The sensitivity for predicting high-grade cervical intraepithelial lesion or carcinoma (CIN3+) was calculated for these women with HC2 or Cervista HPV assays.

Results

In the CPC, HPV positive rates were significantly different between Cervista HPV (4.3%) and HC2 (3.4%, P=0.006) in women who had a Pap result of Negative for Intraepithelial Lesion or Malignancy (NILM). HPV positive rates were similar between HC2 (6.1%) and Cervista HPV assay (6.8%) in women with NILM Pap results in the GYN clinic (P=0.21). In the GYN clinics, HPV positive rates were moderately different between Cervista HPV (25.8%) and HC2 (20.7%) in women with Abnormal Squamous Cells of Undetermined Significance (ASC-US) Pap result (P=0.02). In women with High-grade Squamous Intraepithelial Lesion (HSIL) or carcinoma Pap results, HPV positivity was significantly lower by Cervista HPV (78.8%) than HC2 (92.4%) (P=0.008). However, no significant difference of the sensitivity of HPV to predict CIN3+ was observed between Cervista HPV (81.8%) and HC2 (89.2%) (P=0.30) in women with HSIL/carcinoma Pap results.
Conclusion

In a low-risk cervical cancer screening population, increased HPV positivity by Cervista HPV testing in women with a NILM Pap test may result in a more frequent follow up for women with NILM Pap results. The efficacy of the Cervista HPV assay is marginally lower than that of HC2 in women with HSIL/carcinoma in a cancer surveillance population. Further studies are needed to delineate the efficacy of both Cervista and HC2 HPV assays in this population.
P08-06
SIGNIFICANTLY HIGHER RISK FOR HIGH-GRADE CERVICAL LESIONS IN FOLLOW-UP BIOPSY ASSOCIATED WITH POSITIVE APTIMA HPV TESTS THAN COBAS TESTS

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Background / Objectives

HPV tests and genotyping have been used in clinical risk assessment. The purpose of this study was to analyze the performance of two common HPV testing platforms in risk evaluation for high-grade cervical lesions (HSIL+, including CIN2 and above).

Methods

Between January 1 and December 31, 2016, 4732 Pap tests with biopsy confirmation were analyzed along with HPV tests performed on Cobas (Cobas 4800 system, Roche Molecular Diagnostics, Pleasanton, CA) or Aptima (Hologic/Gen-Probe, San Diego, CA) platforms.

Results

There were 4105 and 627 Pap samples tested on Cobas and Aptima platforms, respectively. Both platforms were highly sensitive for biopsy-confirmed HSIL+ lesions (98% and 96% for Cobas and Aptima, respectively, p=0.51). However, Cobas HPV testing showed significantly higher positive rates for diagnosis of benign (86% vs. 56%) and LSIL (90.5% vs. 66.4%) on biopsy as compared to Aptima. As a result, Aptima HPV testing had a significantly higher specificity for HSIL+ than Cobas (38% vs. 12%, p<0.0001). Overall, performance of Aptima platform was superior to Cobas in detecting biopsy-confirmed HSIL+ due to providing significantly higher positive predictive value (25% vs. 16%, p<0.0001) and overall accuracy (48% vs. 24%, p<0.0001). Aptima hrHPV genotyping also demonstrated a significantly higher specificity for HSIL+ on biopsy than Cobas genotyping measured by either HPV 16/18/or45 (87% vs 73%, p<0.0001) or non-16/18/or45 (51% vs. 39%, p<0.0001) with comparable sensitivity (50% vs. 52%, p=0.68 and 47% vs. 46%, p=0.92, respectively).

Conclusion
Despite Aptima and Cobas platforms offer comparably high sensitive tests for high-grade lesions, Aptima HPV test and genotyping demonstrated significantly higher specificity and positive predictive value than Cobas testing for biopsy-confirmed HSIL+ lesions. The considerable difference may be related to the significant increase in E6/E7 expression following HPV DNA integration in HSIL+ lesions. The significantly higher specificity and overall accuracy of Aptima test and genotyping for HSIL+ lesions may be useful in clinical risk management by identifying high-risk populations.
BACKGROUND / OBJECTIVES

HPV DNA test is performed in a cytology laboratory by trained cytotechnologist as a triage test after borderline cytology or in follow-up after excisional treatment of cervical intraepithelial neoplasia. The aim of the study was to analyze the application of the HPV test in routine cytology laboratory practice in the detection of patients with increased risk of HSIL.

METHODS

We retrospectively analyzed the results of 19,459 HPV tests (Hybrid Capture 2, Qiagen, Germany) performed between 2005 and 2015 and compared with cytological diagnosis on conventional Pap smear. We also analyzed data of initial cytology and HPV test result from 1157 patients in a six-year follow-up period after HPV test was made. A positive outcome represents a histological diagnosis of HSIL +.

RESULTS

Out of the 19,459 HPV tests, 41.9% were positive, of which 21.5% of negative cytology, 39.5% of ASCUS, 71.6% of ASC-H, 77.3% of LSIL, 86.2% of HSIL, 16.9% of AGC and 75% of AIS. Out of 25 cases of cytological diagnosis of cervical cancer HPV test was negative in two histologically verified cervical cancer and in five cases of endometrial cancer. Of 1157 patients with HPV test made in 2009, 652 (56.4%) had abnormal cytology, and 473 (40.9%) had positive HPV test. The mean age of patients was 37 years (range 16-77 years). In the six-year follow-up period histological analysis was performed in 213 patients and verified HSIL + in 173 patients which is 25% of all initial abnormal cytology and 34% positive HPV tests HSIL + was found in 2.5% of patients with initially negative cytology, 8.3% of ASCUS, 10.9% of LSIL, 10% of AGC, 56.5% of ASC-H, 63.5% of HSIL and in 100% of cytological diagnosis of cancer. For all the tested samples, the HPV test showed 94.2% sensitivity, 68.4% specificity, 34% positive predictive value and 98.5% negative predictive value. Reflex HPV testing in the triage of ASCUS showed 86.2% sensitivity, 65.9% specificity, 18.7% positive predictive value and 98.1% negative predictive value. In the six-year follow-up with the initial ASCUS cytology in 8.3% cases verified HSIL + lesions, and with additional triage HPV testing this percentage
rises to 18.7%.

**Conclusion**

The percentage of positive HPV test increases with the severity of cytologic diagnosis. The high negative predictive value confirmed the value of the test in the triage of borderline cytology. Knowledge of cytologist and clinician of the positive HPV test may improve the selection of patients with an increased risk of HSIL lesions.
A HIGHLY EFFICIENT ASSAY FOR DETECTION OF HIGH-RISK HPV E7 PROTEINS IN CERVICAL SAMPLES

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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

A hrHPV E7 sandwich ELISA – recomWell HPV 16/18/45 - was developed for detection of the hrHPV types 16, 18, and 45. Suitable for measurement of E7 protein are liquid-based cytological samples in PreserveCyte.

Results

Sensitivity (CIN2+/CIN3+/CxCa: 36.1/58.3/85.7%), specificity (>98%), positive predictive value (PPV) (CIN2+/CIN3+/CxCa: 59.5/56.8/16.2%) and negative predictive value (NPV) (>97.5%) were calculated across all studies with 1572 clinical samples.

1473 samples were analyzed for validity of E7-based triage for HPV16/18 positive women. 282 women were positive for hrHPV DNA testing and further subjected to colposcopy. For the detection of CIN2+ for HPV16/18 positive women without further triage, sensitivity and PPV were 100.0% and 11.11%, respectively. No triage of HPV16/18 positive women required 9 colposcopies to diagnose one case of CIN2+. The sensitivity of recomWell HPV16/18/45 was 100.0% (meaning that no CIN2+ case was missed) and PPV was 19.75%. The recomWell HPV16/18/45 identified all 16
CIN2+ cases, requiring 43.75% less colposcopies than no triage of HPV16/18 positive women.

**Conclusion**

Detection of hrHPV E7 by ELISA is a feasible method for diagnosing HPV-induced, high-grade cervical dysplasia. Our results support the detection of HPV E7 oncoprotein as a method of triage to colposcopy for HPV16/18 positive women (instead of no triage) in the framework of a screening program based on primary HPV screening with HPV 16/18 genotyping.
Background / Objectives

The Xpert HPV test (Cepheid) detects HR-HPV 16, 18-45 and 3 groups of other HPV types (P3 : 31-33-35-52-58, P4 : 51-59 and P5 : 39-56-66-68) by PCR, including the detection of a reference gene, confirming an adequate cellularity. To date this technique hasn’t yet been evaluated for cervical smears fixed in BD-SurePath transport medium.

The HC2 (Qiagen) HPV test targets 13 HR-HPV types (no HPV 66 detection).

The aim of the study was to evaluate the performance of the Xpert HPV test on SurePath fixed cervical smears by comparing it to the HC2 HPV test as reference method.

Methods

We tested 110 consecutive SurePath ASCUS smears by HC2 and Xpert HPV using the residual cell pellets after BD-autocyte® PAP cytology.

Samples with discordant results were submitted for detailed genotyping by Inno-LIPA PCR Version Extra II (Fujirebio).

We further evaluated the repeatability (5x) with different technicians on 2 different modules(4 HPV+ and 1 HPV- smears), the stability across time of one positive smear at 1, 7, 14 and 28 days and the reproducibility between the initial vial and the residual cell pellet (35 samples).

Results
Of the 110 HC2 tested ASCUS smears, 57 were HC2 HR-HPV+, 53 were HC2 HR-HPV-. The overall concordance was 89.1%, the negative concordance was 96.2% (two HC2- smears were Xpert HPV+) and the positive concordance was 82.5% (of 57 HC2+ samples, 10 were Xpert HPV-).

Among these 10 HC2+/Xpert HPV- smears genotyping revealed: one HPV 39+, one HPV 52/66/70+, one HPV 33/53+, one HPV 33/35/51/58/53/70/52/44+ and six not targeted HPV types: four HPV53+ samples, two of them coexpressing HPV54 or HPV67, one HPV67+ and one not yet classified HPV type.

Among the HC2-/Xpert HPV+ cases one was HPV 18/66/70/62+ and one HPV 16/51/62+.

The repeatability according to different technicians/modules and across time was 100%. The reproducibility between initial vials and cell pellets was 97%.

When we look at the 10 HC2+/HPV- cases and considering the targeted HPVs, after genotyping only 4 Xpert HPV- cases were true false negatives, all of them with a RLU/cut off ratio < 5 considered at low risk for CIN2+(1). None was false positive.

The other 6 HC2+/Xpert HPV- concerned non targeted HPVs due to cross-reactions of HC2 with low risk and potentially HR-HPVs. Colposcopy was normal for 5 of these 6 patients, 2 of them with a biopsy within normal limits.

Two HC2-/Xpert HPV+ smears were probably related to insufficient sample quantity.

Our global, negative and positive concordances (89.1%, 96.2% and 82.5%) show a performance quite similar to the ones reported for the PreservCyt (Hologic)(2,3).

**Conclusion**

The SurePath transport medium is suitable for routine HPV testing with the Xpert HPV.

**References**


HR-HPV TESTING ON FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) SAMPLES: PERFORMANCE EVALUATION OF XPERT® HPV VERSUS PCR INNO-LIPA® EXTRA II GENOTYPING AND P16 IHC ON 28 HEAD AND NECK CARCINOMAS

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Background / Objectives

Today the HPV status of oropharyngeal carcinomas is a prognostic marker impacting treatment.

The Xpert®HPV test (Cepheid) detects HR-HPV 16, 18-45 and 3 groups of other HPV types (P3: 31-33-35-52-58, P4: 51-59 and P5: 39-56-66-68) by PCR including the detection of a reference gene, confirming the presence of an adequate number of cells. To date only one publication used this technique on FFPE tissue sections (1).

The aim of the study was to evaluate the Xpert HPV test on FFPE tumor samples by comparing it to the INNO-LIPA PCR Version Extra II (Fujirebio) genotyping and p16 immunohistochemistry (IHC) as reference methods.

Methods

The Xpert HPV-test on FFPE was validated on six 4 µm FFPE tissue sections of ten cervical biopsies (5 CIN2/3 and 5 within normal limits) and 5 anal biopsies with AIN 2/3 compared to p16 staining.

Tissue sections were deparaffinised, followed by a simple lysis (ATL lysis buffer, Qiagen) with proteinase K for 4 hours, heated for 1 hour at 90°C then diluted in 1 ml ultra-filtered water and processed.

Four of the positive samples were tested at different lysate dilutions: 1/2, 1/4, 1/8, 1/16 and 1/32.

We then performed Xpert HPV tests on 28 FFPE tumor samples of head and neck carcinomas formerly tested by INNO-LIPA PCR (13 HR-HPV positive and 15 negative) and compared them to the IHC expression of p16. Only an intense diffuse staining of > 80% of tumor cells was considered positive, patchy staining was considered negative.
Results

All high grade cervical and anal neoplasias were Xpert HPV and p16 positive.

For the 4 diluted CIN2/3 lysates, HR-HPV was still detected at a dilution of 1/32 and two 4µm sections showed to be sufficient.

Of the 28 head and neck tumor samples, 13 were INNO-LIPA HR-HPV positive, 15 were negative. The overall concordance was 85.7% with a negative concordance of 93.3% and a positive concordance of 76.9%.

One Inno-Lipa negative sample was Xpert HPV as well as p16 positive. Among 13 INNO-LIPA positive samples, 3 were Xpert HPV negative, p16 staining was negative favoring a non viral carcinogenesis. These cases could reflect a possible latent HPV infection in the vicinity of the tumors, detected by the INNO-LIPA PCR.

Conclusion

In all samples tested the overall concordance between Xpert HPV and p16 IHC was 100%.

Xpert HPV testing is feasible without DNA extraction even on very small tissue samples and may be considered as a valuable method for the detection of HR-HPV in FFPE tissues.

References

Background / Objectives

Human papillomavirus (HPV) is a well-studied etiologic agent for cervical cancer dysplasia and neoplasia. Worldwide the most common HR-HPV are 16/18, and approximately 70% of cervical cancer are due to these genotypes. The HPV test, as primary method of cervical cancer screening, decreases the incidence of invasive carcinoma in 60-70%, and its performance is superior when compared to cytology, showing a negative predictive value very close to 100% [Consenso SPG, 2014]

HR-HPV screening is highly sensitive, but specificity depends on subsequent evaluation strategies and screening frequencies. Various methods are available for HPV detection and FDA-approved assays are on the market using either signal or target amplification methodologies. The aim of this study was emphasizing the overall performance of the methods used by Centro Medicina Laboratorial Germano de Sousa (CML GS) and correlate the results with cytological examinations in a 5 years sample population from Hospital Cuf Descobertas using different molecular platforms.

Methods

From January-2012 to Decemeber-2016 were analyzed more than 6000 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.

Conclusion

This study will contribute for a better understanding of the wide spectrum of HPV infection and provide held information to establish interpretation algorithms in diagnostic management. The results obtained to the incidence and most frequent type of HPV were in agreement with the results particularly described by the Portuguese Society of Human Papillomavirus [Consenso SPG, 2010].

The most frequent HPV HR type in Portuguese population is HPV53, where the malignancy rate is not as high as 16/18, but a shift possibility can occur with
universalization of vaccine. The hc2 and 16/18-Cobas accomplished concordance in false positive rate, detection rate and specificity. However some statistically significant differences were seen, particularly 16/18-Cobas yield lower false negative rate for Abnormal Cytological results, subsequently higher negative predictive value. For those reasons 16/18-Cobas testing should be better for triage. When choosing of any HPV assay for cervical screening, quality control and quality assurance aspects should also be considered, in order to maximize the potential of each method in the diagnostic algorithm.
In-house liquid based medium validation for hrHPV detection with Hybrid Capture 2 (HC2), QIAGEN

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Background / Objectives

In Slovenian cervical cancer screening program ZORA, Qiagen HPV test Hybrid Capture 2 (HC2) is used for a triage of women with low-grade cytology and as test of cure since 2011. For these analyses cervical samples are collected in Standard Transport Medium (STM), Qiagen. The major disadvantages of STM are poor preservation of cell morphology and high cost. In-house liquid based medium (LBM) is already extensively used in routine laboratory practice for immunocytochemical and molecular tests at the Institute of Oncology Ljubljana and in some other Slovenian laboratories. It is cheaper than STM and enables both morphological and molecular analysis. Routine use of in-house LBM in the national cervical cancer screening programme would allow a single sampling procedure for both liquid based cytology and HPV testing. However, the new medium might affect results of hrHPV analysis. The aim of the study was to compare and validate in-house LBM toward the STM for detection of hrHPV with HC2.

Methods

In 183 women (aged from 20 to 64 years, 38.3 on average) referred to colposcopy two cytological cervical samples were taken prior colposcopy by physician at the colposcopy clinic. First cytological sample was taken with endocervical brush and Ayer spatula for PAP smear, after that both devices were stored in in-house LBM; second was taken with Qiagen brush and store[d in STM. Cytological samples in different media were analysed on hrHPV by HC2 at cut-off value RLU/CO = 1.0. Results were compared and then validated against the worst histology result from the screening registry within one year since the samples were collected.

Results

HPV-positivity rate was higher in in-house LBM (135/183, 73.8%) than in STM (128/183, 69.9%). Agreement of results was excellent (174/183, 95.1%; Kappa = 0.879 (p < 0.001). Test performance was comparable, however STM had slightly higher sensitivity for CIN2+ (95.5 vs. 96.6%) as well as specificity (46.8 vs. 55.3%), NPV (91.7 vs. 94.5%) and PPV (63.0 vs. 67.2%). Among 9 discordant cases, 1 case was HPV negative in in-house LBM but positive in STM and 8 cases were HPV positive in in-house LBM but negative in STM. 89/183 (48.6%) women had CIN2+.

Conclusion
Comparable HPV-positivity rate, agreement of HPV analyses, sensitivity, specificity, PPV and NPV for CIN2+ between the two media suggest that in-house LBM could be used for hrHPV testing instead of STM to reduce costs and preserve morphology. However, larger prospective study on screening population has to be performed to confirm this assertion.
ONCLARITY IN THE DIAGNOSIS OF PATIENTS WITH CERVICAL LESION: COMPARISON WHITH HC2 AND LINEAR ARRAY

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Background / Objectives

Many methods are available today for HPV testing: they differ for technology, targets, and information on the genotypes detected. A key issue is represented by the differences in analytical and clinical sensitivity, especially in case of genotyping. Aim of this work was the evaluation of BD Onclarity HPV assay in a group of patients referred to the European Institute of Oncology of Milan for a cervical lesion.

Methods

One hundred sixty-seven women scheduled to be conservatively treated for a CIN2+ lesion were enrolled. For all the patients a cervical sample was taken before treatment, and the results of Qiagen Hybrid Capture 2 and Roche Linear Array HPV Test, cytology and histology were available. BD Onclarity was performed on a leftover aliquot.

Results

Concordance of HC2 and Onclarity was 92% (150/163), with 13 samples giving discordant results (4 hc2 negative and Onclarity positive – 2 CIN3 and 1 Carcinoma histology- 9 hc2 positive and Onclarity negative – 3 CIN1 and 6 CIN3 histology (2 of which were also Linear Array negative)). Looking at genotyping a complete concordance was found in 75.5% (126/167 of the cases), reaching the 86% when adding the samples partially concordant in case of multiple infections.

Conclusion

This study performed in a group of women with a high prevalence of disease showed a good concordance between HC2 and Onclarity in the cervical samples taken before treatment. Regarding genotyping the comparison with Linear Array confirmed a good concordance between the two methods.
P08-14
Comparison of Seegene Anyplex II HPV 28 detection and Abbott Realtime High Risk HPV test on NOVAprep liquid-based cytology media.

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Background / Objectives

High Risk (HR) HPV DNA testing is a highly sensitive method to screen women at risk of CIN2+ lesions. But many assays and various media for cervical sample collection are available. So, we aimed to compare the Seegene Anyplex™ II HPV 28 Detection and the Abbott RealTime High Risk HPV test on cervical samples collected on NOVAprep® liquid based cytology media (Novacyt).

Methods

Samples were collected on NOVAprep® media from July 2016 to February 2017. Cytology was performed with NOVAprep® liquid-based cytology platform for cervical cancer screening. Samples with ASCUS cytology were routinely tested with the Abbott assay for HPV testing (14 HR HPV detected:16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Among them, 162 samples were randomly selected for testing with the Seegene assay after extraction on Easymag (Biomérieux) following manufacturer’s recommendations. Anyplex™ II HPV28 Detection simultaneously detects 19 HR HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 9 low-risk HPV (6,11, 40, 42, 43, 44, 54, 61, 70), with semi-quantitative analysis (+, ++ or +++). Discordant results were confirmed by testing again both assays at the same time and then will be sequenced by NGS

Results

All the 162 samples have interpretable results as internal control was valid in both assays. Global HR HPV prevalence was more elevated with Seegene assay (49.38%) than Abbott assay (37.65%). Regarding the 14 HR genotypes detectable by both assays, overall agreement between both assays was very good (93%; kappa 0.86). All discordant results were confirmed in second runs (Seegene and Abbott), showing excellent reproducibility of each assay. Among the 10 Abbott HR HPV negative samples detected as HR HPV by Seegene, analysis of Abbott amplification curves showed that HR HPV (other than HPV16 or HPV18) were detected in 2 samples after 32 Ct (detection threshold determined by Abbott). For both samples, semi-quantification from Seegene assay found a small amount of HR HPV (only one +). Among these discordant results, HPV 68 is the less efficiently detected HPV by Abbott
assay (6/10). Small amounts of HR HPV (one +) were also found in three of the four other discordant results. Only one HR positive with Abbott assay was not detected by Seegene assay. Complete analysis of discordant results by NGS is ongoing.

Conclusion

NOVAprep® medium previously validated with Hybrid Capture assay has demonstrated again strong performances in terms of detection of HR HPV and stability of the patient samples with Abbott RealTime High Risk HPV test and Seegene Anyplex™ II HPV 28 Detection assays.

References


P08-15
METHYLATION OF WIF1 GENE AND MICRORNA EXPRESSION IN DIAGNOSIS OF HPV-ASSOCIATED SQUAMOUS INTRAEPITHELIAL LESIONS AND SQUAMOUS CERVICAL CANCER

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Background / Objectives

Study of diagnostic significance of methylation status of WIF1 gene and microRNA expression (Mir 92a, 22, 25) in diagnosis of SIL and cervical cancer.

Methods

Clinical (including colposcopy); fluid cytology with agent staining using BD Sureph T.M. method for immunocytochemical (ICC) examination with p16/Ki67 dual labeling; HPV test by means of RT-PCR method; WIF1 gene methylation; microRNA expression (Mir 92a, 22, 25). Final diagnostic verification of SIL and cervical cancer has been carried out on the basis of histologic examination.

101 patients aged from 18 to 49 years have been tested (average age 32.7±0.5 years).

The 1st group – 31 patients with LSIL; the 2nd group – 26 patients with HSIL; the 3rd group – 12 patients with squamous cervical cancer (SCC); the 4th group – 32 healthy patients negative for intraepithelial lesions or malignancy (NILM).

Results

Correlation analysis of morphological examination and cancer markers p16 and Ki67 has shown direct, strong and significant correlation between these two methods (r-Pearson = +0.7 p = 4.75 x 10-17 for Ki67; r-Pearson = +0.83; p = 1.8 x10-29 for p16).

Correlation analysis between the findings of morphological examination and WIF1 gene methylation has established direct, strong, significant bivariate correlation between these two methods (r-Pearson= + 0.8; p = 7.0 x10-33), besides correlation analysis between microRNA expression has demonstrated direct, significant correlation between MiR92a (r-Pearson = + 0.27; p=0.007).

Conclusion

We have revealed significant correlations between the findings of morphological examination confirming the diagnosis of SIL and squamous cervical cancer; cancer
markers p16 and Ki67; WIF1 gene methylation; and microRNA expression. All above-mentioned methods can be used in complex diagnosis of SIL and cervical cancer.
DIAGNOSTIC EXCISION OF CERVIX IN WOMEN WITH PERSISTENT HPV INFECTION WITH NO FORMER EVIDENCE OF CIN IN CYTOLOGY

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Background / Objectives

A persistent infection with human papilloma virus (HPV) is identified as a main risk factor for cervical cancer. Further investigation with cytology and colposcopy has been shown to have lower sensitivity than HPV-testing to diagnose CIN2+. In this study we performed a diagnostic excision of the transformation zone (TZ) by loop electrosurgical excision procedure (LEEP) in women with persistent HPV infection with normal Pap-smear to evaluate the eventual proportion of histologically confirmed CIN2+ in the specimen.

Methods

We prospectively recruited 91 women with persistent HPV-infection without any abnormalities in cytology. In total 40 women attended a gynecological examination including repeated HPV test, Pap smear, endocervical cytology, colposcopy with biopsies and diagnostic LEEP. Biopsies and the LEEP specimen was subjected to histologic analysis. The HPV test was performed using a multiplex real-time PCR assay (hpViR) as earlier described, which detects the following high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (18 and 45 are detected together, and 33, 52 and 58 as one group).

Results

In 19/40 women the HPV infection still persisted at the study visit and 32% (6/19) of those women had CIN2+ in histology of the LEEP specimens. All the cytological samples were normal and none of the punch biopsies confirmed CIN2+ in these women. Of the 21/40 women who had cleared their HPV infection at the study visit all but one with CIN 1 had normal histology of the LEEP specimen.

Conclusion

Our results highlight the high risk of undiagnosed CIN2+ in women with persistent HPV infection combined with a normal gynecological examination, Pap smear, endocervical cytology and colposcopy with biopsies. In such cases LEEP must be kept as a diagnostic and treatment option, at least in women without future desire for...
pregnancy. Counseling women about the risks and expected effects of the treatment can help them to do an optimal informed choice.
Are non-vaccine replacing vaccine genotypes in young women targeted by vaccination programs? A trend analysis from opportunistic screening in Luxembourg

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Background / Objectives

While widespread human papillomavirus (HPV) vaccination is likely to reduce the prevalence of vaccine types 16 and 18, it remains unknown whether high-risk nonvaccine genotypes will fill this ecological niche. In Luxembourg where the national vaccination program introduced in 2008 achieved a coverage of approximately 60%, the first cohorts of vaccinated girls are now entering opportunistic cervical cancer screening yielding an opportunity to investigate both hypotheses of vaccine type reduction and nonvaccine genotype replacement.

Methods

We extracted HPV test results from a large clinical laboratory in Luxembourg offering HPV genotyping in the context of opportunistic cervical cancer screening. 17901 HPV test results of cervical samples of adult women (mean age 37 y.) during the period January 2010 – June 2017 were assessed in this study. After screening by Hybrid Capture 2 (Qiagen, Germany) assay, positive samples were genotyped using LCD-Array (Chipron, Germany). We compared fractional polynomial prevalence trends of individual 13 high risk HPV (hrHPV) genotypes by logistic regression and the relative contribution of the 13 genotypes over time in young age groups targeted by vaccination (<25 y.) and older untargeted age groups (>=30 y.).

Results

Overall, 3631 samples (20.3%) were positive for hrHPV, including 583 samples (31.8%) in women younger than 25 y. Increasing prevalence over time (p<0.05) were observed for individual genotypes 39, 51, 52 and 59 among women younger than 25 y., but not in women older than 30 y. Among hrHPV positive women younger than 25 y., the relative contribution of vaccine types 16 and 18 dropped from 34% in 2010 to 7% in 2017 (chi2-test, p<0.001). In this age group, the relative contribution of genotypes 39, 51, 52 and 59 increased from 21% in 2010 to 52% in 2017 (chi2-test, p=0.001).

Conclusion

Between 2010 and 2017, a significant change of hrHPV genotype distribution in young women undergoing opportunistic cervical cancer screening occurred in
Luxembourg. Whether these changes represent genotype replacement remains unclear. Studies in other settings are warranted to verify our findings. In any case, the clinical impact for screening of nonvaccine high-risk genotypes deserves further investigation.
NEW SCENARIOS OF HPV SCREENING - GEORGIAN EXPERIENCE

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Background / Objectives

The study aimed to pilot the modern approach to cervical cancer screening program, which means: a) the application of liquid based cytology, and b) human papillomavirus (HPV) genotyping.

Methods

1500 cervical cytology samples and 1800 blood samples have been analyzed in country of Georgia. The cytology samples had been collected and processed by the usage of materials and equipment provided by Hologic. Prepared smears were post-fixed in 96% ethanol and stained accordingly with Papanicolau protocol. The Bethesda 2001 system terminology was employed for reporting and diagnoses of cervical smears. The blood samples have been collected and processed by the usage of reagents provided by Norgen Biotek for the aim to reveal and genotype HPV. The polymerase chain reaction has been performed.

Results

The negative for intraepithelial lesion or malignancy (NILM) category was equal to 1341 cases (89.40%). Other categories in decreasing order were atypical squamous cells of undetermined significance (ASCUS) with 120 cases (8.00%), low grade squamous intraepithelial lesion (L-SIL) with 9 cases (0.60%), high grade squamous intraepithelial lesion (H-SIL) with 2 cases (0.13%), atypical squamous cells, cannot exclude high grade intraepithelial lesion (ASC-H) with 24 case (1.6%) and atypical glandular cells of undetermined significance (AGUS) with 4 case (0.27%). Cellularity was lower in liquid based cytology (LBC) as compared with conventional smears (CS). Also, nuclear overlap was significantly less observed compared to CS. The smear background was notably cleaner and cell morphology was better evaluated in LBC. In terms of Trichomonas and Candida detection, LBC was superior compared to CS. Doderlein lactobacilli were seen in significantly lesser amounts and were mainly situated in close vicinity to the squamous epithelial cells. Due to lack of pretreatment, the degree of inflammation was better assessed in CS.

The prevalence of HPV DNA has been observed in 586 cases (32.56%). In 320 cases (54.61%) have been determined oncogenic (16/18/31/33/53) HPV.

Conclusion

Our experience shows that LBC is superior to CS in the evaluation of cell morphology and detection of certain microorganisms such as Trichomonas and Candida. The
degree of inflammation is better assessed with CS. CISH is effective and easy for implementation method for HPV genotyping on cervical smears. There has been revealed that HPV genotyping is the effective and accurate screening method.

References


AGE DISTRIBUTION AND RISK PROGRESSION OF HSIL AND HIGHER LESIONS IN THE PUERTO RICAN POPULATION IN 2015

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Background / Objectives

Screening patterns are changing constantly and most of them do not take into consideration younger patients. Most of the High-grade Squamous Intraepithelial Lesions (HSIL) carries a higher risk of progression to cervical cancer given that 40% to 50% of HSIL progress. The aim of this study was to demonstrate the importance of giving relevance to these patients and that age is also a factor for the risk of disease progression, since LSIL patients are not aggressively treated in these younger patients.

Methods

A total 1,072 samples with HSIL or higher lesions were obtained from a pool of 32,620 samples with a dysplasia diagnosis out of a total of 227,946 patients from OB/GYN clinics around Puerto Rico in 2015. The samples were rescreened and classified following TBS criteria for HSIL or higher and then divided by age. Out of the 1,072 samples, 186 of them were tested to determine the risk of progression for overexpression of HPV E6/E7 mRNA, using the OncoTect assay test (IncellDxTM), and was classified as positive in cases where >2% of cells showed overexpression of E6/E7 mRNA.

Results

From these samples, 17.3% of the cases were positive using the OncoTect assay test (IncellDxTM). From 1,072 HSIL or higher cytology samples, the age distribution were: ≤25 (16%), 26-35 (34%), 36-45 (22%), 46-55 (12%), and ≥56 (17%), were 50% of the cases fell under 35 years of age, while the progression risk was: ≤25 (17%), 26-35 (38%), 36-45 (23%), 46-55 (8%), and ≥56 (15%).

Conclusion

This study demonstrated that a relevant percentage (50%) of the patients with HSIL or higher lesions was in the range of 35 years or less, showing that this group had the same percentage of progression to that of older patients. Knowledge that a HSIL or higher lesions prevalence in younger women is by no means negligible, transient or productive HPV infection in this group age is prevalent. Although cytology interpretation has its limitations, it is always best to add more knowledge towards qualifying reports. It is necessary to take early care of these younger patients with HSIL or higher lesions and positive for overexpression of HPV E6/E7 mRNA in the
long run, so as to reduce the risk of progression of disease in the Puerto Rican population.
ACCURATE DETECTION OF HUMAN PAPILLOMA VIRUSES BY PNA MEDIATED REAL TIME PCR USING MELTING CURVE ANALYSIS

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Background / Objectives

Cervical cancer, which is caused by infection with oncogenic human papillomavirus (HPV) is the fourth most common cancer among women in worldwide. In particular, viruses 16 and 18 are known to account for about 70% of the causes of cervical cancer. Although the Pap smear is used as a primary method for cervical cancer screening, it is not possible to predict the potential risk of cancer due to the virus because it tests for cell deformation. Therefore, the need for HPV DNA testing for the early diagnosis of cervical cancer is increasing.

Methods

A new peptide nucleic acid (PNA)-assisted melting curve analysis technique is developed. Each genotype-specific PNA probe, which is conjugated with a fluorescent dye and a quencher, is used as a reporter in a real-time PCR reaction. A PNA probe can design relatively shorter binding sequence than DNA probe, so PNA probe can avoid sequence variation position on a gene. Furthermore, PNA probe showed bigger melting temperature difference than DNA probe when reporter probe bound to a single mismatch target. So, sequence variants on a target gene are easily distinguishable using melting curve analysis. Therefore, PNA-based reporter probe is very useful for multiplex detection in real-time PCR platform to identify a target gene with many sequence variants.

Results

We have developed accurate and simple method to detect of HPV types within 3 hrs in one-tube. PNA probe-based fluorescence melting curve analysis technology in a real-time PCR system [PANA RealTyper™ HPV Screening Kit] is possible to detect 16 types of HPV [Genotyping types: 6, 11, 16, 18(Type associated with vaccine prescription) and Screening type: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68(HPV high risk)]. The PNA probes were designed to detect all variant genes for each HPV type. Using standard materials, each type of HPV identified the sensitivity of detection was $5 \times 10^1$ ~ $5 \times 10^3$ copies. And there was no cross reaction with other HPV types.

Conclusion

PANA RealTyper™ HPV Screening kit is easily and rapidly can be detected HPV types, and it will be a useful and efficient method for detection and discrimination of HPV types in clinical diagnosis.
P09-04
COMPARISON OF P16/Ki67 DUAL IMMUNOCYTOCHEMICAL STAINING, HPV TESTING AND CYTOLOGY RESULTS OBTAINED IN THREE CYTOPATHOLOGY LABORATORIES PARTICIPATING IN SLOVENIAN CERVICAL CANCER SCREENING PROGRAM ZORA


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Background / Objectives

Slovenian organized, population based cervical cancer screening program ZORA is cytology based. Women with low grade (LG) cytology are triaged with HPV testing. After the implementation of the program in the year 2003 the incidence of cervical cancer decreased for almost 50%, from 20.7 to 11.4 per 100,000 women (crude incidence rate). Despite the good results the gradual introduction of HPV primary screening is currently discussed in Slovenia and therefore the pilot study was conducted to compare the results of PAP test, p16/Ki67 dual immunocytochemical staining (ICS) and HPV testing within the Slovenian cervical cancer screening program.

Methods

In 129 women referred to colposcopy, cervical smears were obtained for cytological examination, p16/Ki67 dual ICS (CINtec PLUS test, Roche) and HPV testing (Hybrid Capture 2, Qiagen). Each PAP smear and ICS slide was evaluated blindly in 3 laboratories participating in Slovenian cervical cancer screening program by a screener and a cytopathologist in the same way as it is current practice in the program. Cytology was reported according to Bethesda classification and p16/Ki67 according manufacturer recommendations. Sensitivity and specificity for CIN2+ were calculated for LG cytology (ASCUS+), high grade (HG) cytology (HSIL+), p16/Ki67 dual ICS and HPV testing. For cytology and p16/Ki67 dual ICS summary estimates of sensitivity and specificity were calculated for the three laboratories participated in the study.

Results
The sensitivity for LG cytology, HG cytology, p16/Ki67 dual ICS and HPV testing were 69.2% (95% CI 54.1%–81.1%), 44.4% (95% CI 33.6%–55.8%), 88.2% (95% CI 82.7%–92.1%) and 96.8% (95% CI 89.0%–99.6%) respectively. The highest sensitivity was obtained for HPV testing, however the sensitivity for p16/Ki67 dual ICS staining was much better than for cytology. The specificity for LG cytology, HG cytology, p16/Ki67 dual ICS and HPV testing were 67.2% (95% CI 56.4%–76.5%), 93.0% (95% CI 87.6%–96.1%), 73.1% (95% CI 66.5%–78.8%) and 59.1% (95% CI 46.3%–71.0%) respectively. The highest specificity was obtained for HG cytology and p16/Ki67 dual ICS.

**Conclusion**

Our results were similar to the results of other studies and support the idea that the introduction of HPV primary screening with p16/Ki67 dual ICS or cytology triage could give better results than cytology primary screening with HPV triage. However, additional larger prospective study on the screening population must be carried out before the policy of cervical cancer screening program in Slovenia would be changed.
Background / Objectives

As jurisdictions prepare for HPV-based cervical cancer screening, programs cannot ignore operational concerns, such as compliance with colposcopy referral for timely disease detection. Colposcopy programs traditionally prioritize women with high-grade cytological abnormalities. With HPV-based screening and cytology triage, traditional patterns for colposcopy prioritization may need to be re-assessed. We present colposcopy compliance and procedure wait times by referral result from HPV FOCAL, a large primary HPV testing RCT.

Methods

HPV FOCAL compared primary HPV testing with liquid based cytology (LBC) triage (for HPV positives) every 4 years to LBC screening every 2 years. Women 25-65yrs (n=18,948) were randomized to the control (CTRL) and intervention (IA) arms. IA: baseline HPVpos received reflex LBC and were referred to colposcopy if >ASCUS; if baseline HPVneg or <CIN2, exit trial at 48mos. CTRL: baseline ASCUS receive reflex HPV and referred to colposcopy if HPVpos. Baseline >LSIL were directly referred to colposcopy; those baseline LBCneg or <CIN2 were rescreened with LBC at 24mos; if LBCneg or <CIN2, exit trial at 48mos. Both arms co-tested with HPV/LBC at 48mos and referred to colposcopy if positive on either test. To enhance colposcopy compliance and standardization, colposcopy procedures occurred primarily at two high volume clinics.

Results

Overall trial colposcopy compliance was 96% within 12 months of referral compared to the provincial program rate of 86%. At 48mos, where both arms received HPV/LBC co-testing, the shortest median wait times from referral to procedure were in those HPVneg/>HSIL (3.6mos) and HPVpos/>HSIL (3.7mos). Time to colposcopy for >HSIL patients, irrespective of HPV outcome was significantly shorter than other referrals; median wait time, >HSIL: 3.7mos, other: 4.7mos (p< 0.0001). At 48mos, the largest number of CIN2+ (42%) was detected in those HPVpos/NILM. The trend for prioritization by cytology regardless of HPV positivity was also observed in Round 1 of the trial. Trial colposcopy clinics reported confusion regarding how to prioritize HPV positive results accompanied by low grade or normal cytology.
Conclusion

Trial colposcopy compliance for HPV FOCAL was high (96%) compared to program rates (86%), and median wait times for any referral result were less than 6mos. However, longer wait times were observed in those HPVpos/NILM, where the highest burden of CIN2+ was detected at 48 months. As programs plan for HPV-based screening with cytology triage, protocols for prioritizing colposcopy procedures may need to be re-evaluated based on the combination of both HPV and cytology results.
POSITIVE PREDICTIVE VALUE OF HPV SCREEN TESTS AND HPV 16/18 GENOTYPING AT BASELINE AND 48 MONTHS IN THE HPV FOCAL TRIAL

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Background / Objectives

Evidence suggests that positive screening test performance declines upon subsequent screening in women with a history of negative HPV and Pap co-tests1. We examined the positive predictive value (PPV) of the hybrid capture 2 (HC2) and cobas HPV screen tests, and cobas genotyping at the baseline and 48 mo. exit screens in the HPV FOCAL Trial.

Methods

HPV FOCAL is a randomized trial comparing liquid-based cytology (LBC) to high-risk (hr) HPV for cervical cancer screening. Of 9,552 women randomized to the Intervention arm, 9,514 had valid baseline HC2 and cobas results (cobas was blinded at baseline). Round 1 colposcopy referral was based on baseline HC2 positive together with LBC ≥ASCUS or 12 mo. persistent HC2 positivity. At 48 mo. exit, 8,330 women had valid HC2, cobas and LBC results (no blinding at exit), of whom 7,664 were baseline HC2 and cobas negative. Colposcopy referral at 48 mo. was based on HC2 positivity, LBC ≥ASCUS or cobas HPV 16/18 positivity. We calculated PPV for each screen test and for cobas genotyping at both screening rounds.

Results

PPVs for CIN2+ and CIN3+ at Round 1 and 48 mo. exit are shown in the table. At Round 1, cobas HPV 16/18 positive women had significantly higher CIN2+ and CIN3+ PPVs vs. other HPV test results; PPVs for HC2, cobas and cobas non-16/18 hrHPV were similar. At 48 mo. exit, all CIN2+ and CIN3+ PPVs were lower than at Round 1. For cobas HPV 16/18 positive women, CIN2+ and CIN3+ PPVs were higher, but no longer significantly, than other HPV test results.

| CIN2+ and CIN3+ PPV (95% confidence interval) at Round 1 and 48 Mo. Exit Screens |
|---------------------------------|---------------|---------------|----------------|---------------|
| Test result                     | Round 1       | Round 1       | 48 Mo. Exit    | 48 Mo. Exit    |
|                                 | CIN2+ (n=149) | CIN3+ (n=68)  | CIN2+ (n=30)   | CIN3+ (n=9)    |
| HC2+                            | 0.19 (0.17-0.23) | 0.09 (0.07-0.11) | 0.10 (0.07-0.14) | 0.03 (0.01-0.06) |
## Conclusion

At the baseline screen, cobas HPV 16/18 positive women had significantly higher PPVs for CIN2+ and CIN3+ than other HPV test results, but at 48 mo. exit the PPVs for cobas HPV 16/18 positives were no longer significantly higher. As expected, due to lower CIN2+ and CIN3+ prevalence at 48 mo., all PPVs were lower than at baseline. Further research will be required to assess the ongoing utility of screening and triage approaches at subsequent screening rounds following HPV implementation.

## References

P09-07
HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS WITH NEGATIVE HPV TESTING

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Background / Objectives

It is demonstrated that all cervicovaginal squamous cell carcinomas are associated with HPV infection. Based on this evidence, the new international guidelines recommend HPV-DNA testing as the main primary screening method to filter those patients who may present pre-malignant lesions and need to go through further explorations. However, a subset of high-grade squamous intraepithelial lesions is found in women with negative HPV testing.

Our objective with this study is to report the patients in our base data with HPV-DNA negative test and high-grade squamous intraepithelial lesions (HSIL). HPV subtype and histology of the biopsy specimens are reported and taken into account for the final results.

Methods

A retrospective review of the cases identified as citology-positive and HPV-negative testing over a 36 month period at a tertiary care gynecologic center. Two types of testing were compared, enzyme-linked immunosorbent assay (ELISA) detection versus polymerase chain reaction (PCR) of the virus.

Results

A total of 1043 cases (740 patients) meeting the study criteria were selected. 10 patients with high-grade lesions and negative ELISA detection were identified (representing 1.35%), of whom 8 had high risk HPV detected with PCR study (80% of the selected cases).

Of the ten selected patients one of them presented a biopsy positive for vaginal intraepithelial neoplasia (VaIN). The PCR test presented a coinfection with HPV 42 subtype (low risk) and 73 subtype (high risk).

Considering the other nine cervical displasias, three of them presented a biopsy positive for squamous cell carcinoma, with PCR testing positive for HPV subtypes 52, 31, and 73 (high risk subtypes).

Conclusion

As we know, co-testing with the combination of Pap cytology and HPV DNA testing (HPV 16/18) is the preferred cervical cancer screening method for women between
30-65 years old. This combined method has a 5.5% false negative rate in most of the studies.

If we compare the most important international studies with our data we present a lower rate of false negative results using an ELISA testing combined with Pap cytology, and we can overcome this low rate if we complement the testing with a PCR study in the cases of discrepancy between both testings.

We consider that these cases are the ones that could have a greater benefit with the co-testing screening versus an HPV testing alone, as we would be able to compare the citology results with the DNA testing so as to avoid the loss of control and study of those patients with high grade lesions and negative HPV testing.
P09-08
RANDOMIZED HEALTH CARE POLICY EVALUATION
OF ORGANISED PRIMARY HPV SCREENING OF
WOMEN AGED 56-60


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Background / Objectives

To implement and reliably evaluate primary HPV screening in an established and routinely running organized, large-scale population-based screening program.

Methods

Participants: Resident women in the Stockholm/Gotland region of Sweden, aged 56-60 years were randomized to either i) screening with cervical cytology, with HPV test in triage of low-grade cytological abnormalities (old policy) or ii) screening with HPV testing, with cytology in triage of HPV positives (new policy).

Outcome: The primary evaluation was the detection rate of cervical intraepithelial neoplasia grade II or worse (CIN2+).

Results

During January 2012 - May 2014, the organized screening program sent 42752 blinded invitations with a pre-booked appointment time to the women in the target age group. 7325 women attended in the HPV policy arm and 7438 women attended in the cytology arm. In the new policy, the population HPV prevalence was 5.5%, using an accredited HPV test (Cobas 4800). HPV16 prevalence was 1.0% (73/7325) and HPV18 prevalence was 0.3% (22/7325). In the HPV-policy arm, 78/405 (19%) HPV-positive women were also cytology positive. There were 19 cases of CIN2+ in histopathology, all among women who were both HPV-positive and cytology-positive. The PPV for CIN2+ in this group was 33.3% (19/57). In the cytology policy, 153 women were cytology positive and there were 18 cases of CIN2+ in histopathology. Both the total number of cervical biopsies and the number of cervical biopsies with
benign histopathology was much lower in the HPV policy (49 benign, 87 total versus 105 benign, 132 total).

**Conclusion**

Primary HPV screening had a similar detection rate for CIN2+ as cytology-based screening, already before follow-up of HPV-positive, cytology-negative women with new HPV test and referral of women with persistence.
EVALUATION OF THE IMPACT OF THE hr-HPV BASED CERVICAL CANCER SCREENING: RESULTS OF A FOUR-YEARS EXPERIENCE IN A SINGLE SCREENING CENTER OF ITALY.

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Background / Objectives

The knowledge on the etiological role of high-grade Human papillomavirus (hr-HPV) have caused a radical change in the cervical cancer prevention program by introducing hr-HPV test instead of PapTest as a primary test in the screening program. The aim of this study was to evaluate the impact of the hr-HPV test in the cervical cancer screening in the Latina district (Italy) over a four-year period.

Methods

The population was divided in two groups: the hr-HPV as primary test was performed only on women aged 35-64, followed by a PapTest as triage, while women aged 25-34 were invited to perform only a PapTest.

Results

5.6% of women presented with a positive hr-HPV test and 4.1% presented with a positive PapTest. In the group of women aged 25-34, the hr-HPV test was used as triage for women with ASCUS and 69.9% presented with a positive test. The PPV for high cervical intraepithelial lesions (CIN2+) was higher in women aged 35-64 (9.9% vs 6.9%), while the DR for CIN2+ lesions was higher in young women (2.4 ‰ vs 1.2 ‰). Moreover, we found that 52.5% of women hr-HPV+/PapTest- resulted hr-HPV+ at 1-year recall and the DR for CIN2+ lesions of this population was very low (0.27‰).

Conclusion

Our data confirms that the application of Italian guidelines showed high level of performance; moreover, our data confirms that the application of hr-HPV test in the management of ASCUS leads to a decreased of inappropriate colposcopy due to transitory infection in young women. Finally, because of the small number of CIN2+ lesions, it may be useful to extend the period of follow-up for women hr-HPV+/PapTest- to reduce the number of unnecessary colposcopies due to transitory infections.
ORGANIZED CERVICAL CANCER-SCREENING PROGRAM IN BRAZIL: BARRETOS EXPERIENCE IN 18 MUNICIPALITY OF SÃO PAULO STATE


Barretos Cancer Hospital (Brazil)

Background / Objectives

Background: Cervical cancer remains as an important problem for public health authorities in developing countries due to the high rates of incidence and mortality. This malignancy is the second most common cancer among women worldwide, accounting for more than 520,000 new cases and the deaths of approximately 270,000 women, annually. Estimates by the Brazilian National Cancer Institute (INCA) indicate an incidence of 16,340 new cases of cervical cancer for 2016 in Brazil. Objectives: To demonstrate the implementation of an organized cervical screening program in low-resource settings supported by Barretos Cancer Hospital (BCH) initiative to implement.

Methods

Methods: We developed an organizational, laboratorial and human resources training necessary to administrate the program. A computational program to report all epidemiological, clinical and laboratorial findings, and also to trace all necessary informations to periodically recruit the women for regular screening was developed by the BCH. Women from rural and remote areas were screened in mobile units.

Results

Results: More than 160,000 Pap tests were analyzed and 2,900 colposcopy examinations were performed in one single year. Importantly, from 2011 to 2015, 89.4% of all carcinomas were detected at clinical stage in situ carcinoma and I, and only 5% at stages III and IV. In 2014, e.g., 1,130 patients were referred for colposcopy: 98% of the patients from Barretos region attended the call; 97.1% of the patients from other regions attended in Public Health Ambulatories, and 74% attended in Mobile Units from other Brazilian States.

Conclusion

Conclusions: Since the organized system was implemented, 98% of women attended the recall for colposcopy. However, the main restriction of our program for prevention cervical cancer is still the lack of ideal coverage for this agenda. We did not reach yet the 70% of the women target for this proposal as recommended by the international standards.

References

P09-11
CERVICAL SCREENING AND RISK ASSESSMENT USING MULTIPLEXED PROTEIN ASSAY

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Background / Objectives

Cervical cancer, the second most common cancer occurring in women worldwide, is the result of infection by sexually transmitted, high risk strains of the human papilloma virus (HPV)[1]. The persistence of infection and subsequent integration of HPV into the human genome results in a number of molecular and cellular changes, which override normal growth control leading to sustained cell cycle progression and subsequent transformation to cervical cancer. Continued discovery of the molecular mechanisms engaged in malignant transformation of the cervical mucosa has lead to the emergence of several candidate cervical cancer biomarkers that could be used to identify HPV-positive patients at the greatest risk for developing cervical cancer and could one day provide the opportunity to identify those patients who are most likely to benefit from therapeutic intervention.

Methods

We present a description and quantitative means for detection and prognostic risk assessment of cervical disease, CIN II or above using multiplexed proprietary biomarkers. Histological and immunological evidence and modeling for several protein markers against disease and normal patient samples is discussed and proposed as a basis for a multiplex quantitative laboratory system and eventual point-of-care (POC) approach to extend cervical screening to underdeveloped regions.

Conclusion

Multiplexing of protein-based biomarkers for cervical disease can improve sensitivity and specificity of cervical screening, providing opportunities for identifying patients at high risk, while expanding the reach of cervical care to areas of greatest need.

References
Background / Objectives

Cervical cancer is considered an important public health problem especially in developing countries. In Brazil, some populations, for instance the women who live in rural areas, are most vulnerable to the absence of screening organized programs that can provide an extensive coverage and follow up of suspect cases. The possibility of less invasive tests based on self-sample and performed in a domestic environment can increase acceptance rates on screening tests in comparison to the conventional Pap smear. The aim of this study was to evaluate the feasibility of a cervical cancer screening strategy in women who lived/worked in rural areas by using self-sampled tests to detect HPV offered by the Military Police Team, which is responsible to take care of these regions.

Methods

We performed a cross sectional study with prospective collection data from February 2015 to June 2016. The study was developed by Prevention Department of Barretos Cancer Hospital, Barretos, Brazil in partnership and financed by AMIGOH (Einstein’s friends of Oncology and Hematology) which is one arm of the Albert Einstein Benefit Society. We enrolled 386 woman who were employed or lived in rural areas of Barretos, between the ages of 25 and 78 years old (average=41,3). It was offered to the participants the possibility of self-sampled tests to detect high risk HPV which were after processed using Cobas 4800® System (Roche Diagnostics, Laval, Quebec, Canada). This system can provide the detection of 14 high-risk HPV types in a single analysis. The test was offered to each participant by one member of the Military Police (female soldier). All woman with a positive result (HPV positive) were invited to a colposcopy evaluation.

Results

95,6% of woman interviewed accepted the study and performed the self-sample. The main reasons to refuse the study were: “being afraid of getting hurt” and “not consider herself able to perform the test”. In a total of 340 woman with a valid test, 45 (13,2%)
had a positive result for HPV infection of at least one subtype. The combination of the results (colposcopy evaluation and the pathologic results for HPV positive participants) detected 46.3% benign findings, 41.5% CIN1, 7.3% CIN2/3 and 4.9% with invasive squamous cell carcinoma.

Conclusion

The high acceptance of self-sampled test, even when not offered by health professionals, showed the potential of this strategy as a complementary instrument on cervical cancer screening, mainly to populational groups non adherent to the conventional strategies.

References

HPV DNA SELF-SAMPLING OFFERS A VALID TOOL FOR CERVICAL CANCER SCREENING IN KINSHASA, THE DEMOCRATIC REPUBLIC OF THE CONGO

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Background / Objectives

OBJECTIVES. To offer women in Kinshasa an alternative tool for cervical cancer screening by self-sampling with detection of high-risk (hr) HPV DNA.

Methods

A total of 190 women living in the municipality of Bandalungwa, aged 29-73, participated in the test. Women were individually informed at home by health care workers about the purpose of the test, the procedure, the follow-up, and eventual therapy. All interventions were free of charge. Samples were collected using EvalynR Brush (Rovers Medical Devices), and were analyzed by the Abbott RealTime High Risk HPV assay in the Laboratory of Molecular Microbiology in Ghent, Belgium. In total 187 samples were analyzed.

Results

Application of the Abbott assay resulted in 170 valid hrHPV determinations. hrHPV could be detected in 19% of these samples. HPV16 and HPV18 were present in 16% an 6% of the hrHPV-positive samples, respectively. The prevalence of hrHPV in this test was about five times higher than the prevalence of (pre)cancerous lesions (Bethesda grade LSIL and higher) found with cytology in comparable groups of women in Kinshasa (1,2). The finding of the rather low percentages of HPV16 and HPV18 is in agreement with earlier findings published for women with HIV-negative or unknown HIV serology (1,2).

Conclusion

Self-sampling is a valuable tool that facilitates access to cervical cancer screening in Kinshasa. Further research is needed to resolve the origin of differences between cytology and HPV DNA results.

References

FOR HIGH-RISK HPV TESTING THE SENSITIVITY OF A URINE SAMPLE EQUALS THAT OF A SELF-COLLECTED VAGINAL SAMPLE

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Background / Objectives

Increasing focus has been added toward self-collected samples as a means to increase the participation in the screening programs for cervical cancer. Urine samples have also been tested for this purpose, but the knowledge on performance is still quite sparse.

In this study, the high-risk HPV status of a self-collected (SC) vaginal sample and a urine sample is being compared to a concomitantly physician-taken liquid-based cytology (LBC) sample. The results of the three HPV tests are being compared to the histological diagnosis of biopsies taken in parallel.

Methods

Women referred to colposcopy at the gynecological departments at Lillebaelt Hospital and Odense University Hospital is being in-rolled in the study. A total of 300 women will be included.

A urine sample and a SC vaginal sample using the Evalyn Brush are performed by the women after a short instruction and just before the medical examination. At the colposcopy an LBC sample and biopsies are taken by the gynecologist. The urine, SC vaginal sample and LBC sample are analyzed for the presence of high-risk HPV using the Cobas HPV test, Roche. The biopsies is diagnosed by a pathologist and used as the gold standard.

At this point 70 women have been included in the study.

Results

Urine and a SC vaginal sample are available from all women, while data from LBC and biopsy samples are currently available from 60 and 58 women, respectively. The results of the HPV test are distributed as follows:

For urine and the SC vaginal samples, 46 were identified as positive in both sample types and 19 as negative. The concordance was 93 % (65/70). For SC vaginal
samples and LBC samples the agreement was 87% (52/60), while for urine and LBC a concordance of 80% (48/60) was found.

The sensitivity of the three sample types was the same (94% and 100% for CIN2+ and CIN3+, respectively). For the specificity differences was observed. At CIN2+ the specificity was 38%, 43% and 57% for SC vaginal samples, urine and LBC, respectively. For CIN3+ the specificity was 33%, 38% and 53% for SC vaginal samples, urine and LBC, respectively. The differences are not statistically significant at this point.

Among the women one adenocarcinoma was identified and for this patient all three sample types were HPV positive.

Conclusion

These initial data indicate that the sensitivity of a urine sample is equally good as a SC vaginal sample and comparably to the physician-taken LBC samples. Further data are needed in order to determine whether the specificity of a urine sample and vaginal samples is significantly lower than a LBC sample taken by the physician. The updated data will be presented.
A PILOT STUDY OF COMMUNITY BASED SELF SAMPLING FOR HIGH RISK HUMAN PAPILLOMAVIRUS TEST IN CHINESE POPULATION

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Background / Objectives

Cervical cancer ranks as the fourth most common cancer worldwide in women, and it is the eighth most frequent cancer in Chinese population. High risk (HR) human papillomavirus (HPV) is one of the major causes of cervical carcinoma. More than 99% of cervical cancer cases are related to HPV genital infection, which 69.1% of invasive cervical cancer in Chinese population are attributed to HPV HR-subtype 16 or 18. Cervical cancer screening coverage in female population of aged 30-59 years is only 20.9%. This low coverage attributes to the risk of cervical cancer development upon persistent HPV infections. In this study, we therefore investigate the reliability of vaginal specimen collection by self-sampling device (Qvintip, Aprovix) in Chinese population.

Methods

Women aged 30±7.3 years (mean±SD, n=281) attending local health clinic between March 2017 and June 2017 were enrolled in this study. Vaginal specimens were obtained by self-sampling device Qvintip with instructions provided. The specimens were subjected to DNA extraction followed by HR HPV DNA screening using commercial Real Time qPCR test kit.

Results

Among the 281 self-sampling devices collected, observable vaginal fluid was observed in 99.3% of the Qvintips (279/281). β-globin and another housekeeping gene were used as endogenous controls for the presence of DNA. The positive rate for internal control was 98.6% (277/281). The overall infection rate of HR-HPV was 10.8%.

The self sampling device had good acceptability with easy process of specimen collection. The drying and short-term storage (2 weeks) of vaginal fluid on Qvintips did not hinder the reconstitution of specimens for analyses. This provides the possibility of self-sample taking at home which could be suitable for remote area population and enable clinical screenings in laboratories later on. Hence, the value of self-sampling at home should be further investigated.

Conclusion
Community based self sampling was a reliable way for vaginal fluid collection for HPV DNA screening. Implementations of HPV self sampling using Qvintip for the responder population as a primary screening tool is highly achievable.
THE COST-EFFECTIVENESS OF HPV SELF-SAMPLING FOR NON-ATTENDERS IN A DANISH CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

Cervical cancer screening programs are responsible for declining rates of cervical cancer mortality around the globe. However, incidence rate of cervical cancer remains high in un-screened women not attending screening. In the Capital Region of Denmark (RegionH), 24% of women remain unscreened, accounting for 50% of cancers in the region. To address this challenge, the Copenhagen Self-Sampling Initiative (CSI) pilot program was initiated to test the effectiveness of HPV self-sampling as a strategy to reach unscreened women. The pilot successfully demonstrated that 30% of unscreened women could be brought into the screening program2.

Based on the results of the CSI pilot, the RegionH is now implementing self-sampling as a general offer to screening non-attenders over a period of 3 years. A health economic model was developed to predict the cost-effectiveness of this initiative.

Methods

An Excel-based budget impact model was constructed and calibrated with data from the published literature for Denmark’s population, HPV prevalence, and outcomes. Costs were calculated based on direct data from the CSI pilot2,3. For ages 30–59 years, the model compared two identical cytology primary screening algorithms, of which one offered self-sampling.

Results

At full implementation, over 1 screening cycle, the RegionH could expect to bring an additional 32,050 unscreened women into the screening program utilizing self-sampling. Through this additional coverage, 8% more ≥CIN2 and 32% more cancers would be detected. Based on previous analysis in Denmark, it’s expected that 16% of these CIN2 and CIN3 cases, if left untreated, would progress to cancer1. The total costs of the self-sampling program would be an incremental €21,861. This translates to €14 per woman brought into the regular screening program, €1,728 per ≥CIN2 detected, €21,861 per cancer detected, and €11,733 per cancer avoided.

Conclusion
As self-sampling is currently being rolled out in the RegionH, it’s important to understand the cost-effectiveness of this strategy. This analysis demonstrates that self-sampling is a cost-effective strategy to increase coverage of cervical cancer screening programs, improving outcomes for patients.

References

EVALUATION OF THE ROCHE COBAS® 6800 HPV ASSAY WITH COLLI-PEE® COLLECTED, UCM PRESERVED URINE.

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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 aim of this study was to check compatibility with Colli-Pee® collected, UCM preserved urine and compare performance of the Roche Cobas® 4800 and 6800 HPV assays. In addition, a pilot on impact of different preservatives on HPV detection onto the cobas® 6800 System was conducted.

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 postal 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 (Dialex, Heidelberg, Germany). We further tested 15 urine samples of which 10 were previously positive for HPV 16 and/or 18. These samples were stored for 3 days at RT without preservative, with UCM or with Roche preservative.

Results

This pilot study demonstrates that the Roche cobas® HPV 6800 assay performs well with the Colli-Pee® collected, UCM preserved urine samples. All 44 samples were positive for the Roche beta-globin internal control. Comparing CT values of the cobas® 4800 HPV assay with the cobas® 6800 HPV assay showed that lower CT values were observed for the IC control as well as for the HPV 16, HPV 18 and other HR HPV types in the cobas® 6800 System. Compared to cobas®4800 HPV assay an additional 7 samples were positive for HPV 16.

A correlation between the Ct (cycle threshold) values obtained with Cobas® 6800 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.
The impact of preservative was most noted on the Internal Control, 5 of the 15 samples without preservative were reported invalid.

**Conclusion**

We confirm that the cobas®HPV 6800 assay is compatible with Colli-Pee® collected, UCM preserved urine. The analytical sensitivity seems to be increased compared to the cobas® 4800 system. The importance of a preservative is reconfirmed.
Background / Objectives

Background: The ASC-US cytological result accounts for more than half of the abnormal cytology results. The detection and typing for HPV DNA is effective and beneficial in the management of ASC-US citology results, because the HPV type have the relationship with the severity of the squamous intraepithelial lesions. Objective: The aim of the study was to analyze the effectiveness of the HPV-DNA tests in the initial approach of women with ASC-US cytology.

Methods

Methods: A cross-sectional cohort study was conducted with 100 women from the city of Rio de Janeiro with ASC-US cytology using liquid-based cytology, a colposcopy procedure and HPV DNA testing.

Results

Results: The median age was 43 years. In 50% of the women were HPV positive. HPV genotyping test results showed that HPV types 51 and 16 were most frequent. 74% (37/50) of the women were infected with only one type, and 26% (13/50) were coinfected by two or more types. Most (60,9%) of the women with normal cytology were HPV negative, while all the women with severe cytology (LSIL, ASC-H, HSIL) were HPV positive. Most (64,1%) of the women with a normal colposcopy were HPV negative, while all of the women with an abnormal colposcopy (LSIL e HSIL) were HPV positive. There was cyto-colposcopic concordance among 85% (59/69) of the women both tests normals. Only 30% (6/20) of the women with ASC-US cytology had an abnormal colposcopy. However, it was noted that cyto-colposcopic concordance was obtained in 54,5% (6/11) of the women with severe cytology (ASC-H, LSIL, HSIL). Four women underwent biopsies with histological findings of NIC2 (2 cases), NIC3 (1 case) and microinvasive carcinoma (1 case).

Conclusion
Conclusion: Based on these data, it can be concluded that HPV DNA testing in the initial screening of women with ASC-US cytology may be an effective strategy.

References

A comparison study of the INNO-LiPA and the Linear Array HPV genotyping tests

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Background / Objectives

In a routine setting we analyzed DNA from 100 samples of various tissues with the automated procedure using the INNO-LiPA HPV Genotyping Extra II kit (Fujirebio, Malvern, PA, US) and compared the results with the performance of the manual, highly validated HPV genotyping protocol for Linear Array (Roche Diagnostics, Pleasanton, California, US).

Methods

Both methods are line probe assays based on PCR amplification of part of the L1 region of the human papillomavirus (HPV) genome. INNO-LiPA HPV Genotyping Extra II employs SPF10 primers and is designed for the identification of 28 different genotypes where the hybridization steps are performed on the Fujirebio automated platform. In contrast, the Linear Array targets 37 genotypes using PGMY09/11 primers and the hybridization steps are highly manual.

Results

Based on our study, the two methods are highly comparable with 80% of the cases exhibiting complete agreement. This agreement was higher for single infections than for multiple infections. Ninety two percent of all samples were at least in partial agreement (concordant or compatible), however this number was higher for cervical (97%), than non-cervical tissues (80%). This discrepancy is best explained by the increased number of failed assays and/or lower sensitivity of the Linear Array compared to the INNO-LiPA method when FFPE samples are genotyped. Better performance of INNO-LiPA on often degraded FFPE samples is a logical outcome of its primer design, since Linear Array requires nearly 7 times longer DNA fragments for potential identification of HPV genotypes, than INNO-LiPA does. Moreover, Linear array was inferior in detecting HPV 52, probably due to its shared probe design.

Conclusion

Overall, the two methods are highly comparable regarding performance of HPV genotyping and although INNO-LiPA kit is more expensive, it requires substantial less hands-on work in addition to being superior in genotyping of low quality DNA often characteristic of FFPE specimens.
EVALUATION OF THE PERSISTENCE OF HPV GENOTYPES IN WOMEN TREATED FOR CIN2+ LESIONS

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Background / Objectives

The follow-up of women treated for CIN lesions includes HPV testing and cytology, with different schedules depending on different guidelines. Today it is not yet clear if looking at the persistence of the genotype identified at baseline (before any treatment) would be of help in selecting those women who may present a relapse, while reassuring those cured by the surgical conservative treatment. Aim of this study was the evaluation of the utility of using a test giving an extended genotyping in the follow-up of patients treated for CIN2+ lesion.

Methods

One hundred and sixty-seven women scheduled to be conservatively treated for a CIN2+ lesion were enrolled. For all the patients a cervical sample was taken before treatment and at first follow-up visit. In all the patients the results of histology performed at baseline and at relapse (when occurred) was available. The presence of HPV DNA was evaluated with the BD Onclarity HPV assay, which allows an extended genotyping, detecting HPV16, 18, 31, 45, 51 and 52 in single, and HPV 33/58, 35/39/68 and 56/59/66 in pool. In all the patients results of cytology, hc2 and Linear Array were also available.

Results

Of the 167 patients, 161 had the Onclarity performed also at the first follow-up visit. A negative HPV test was found in 120 women, while 41 tested positive (25.5%): 14 cases presented different genotypes from baseline, while 27 of 41 (65.8%) showed fully or partially persistence. Nine patients (5.4%) relapsed and Onclarity was performed at baseline and follow up in 7 of them: 6 had persistence of the same genotypes, while 1 patient tested negative not only with Onclarity but also with hc2. In this patient cytology was HSIL, and Linear Array HPV test revealed the presence of HPV18 and 73 at baseline, with the persistence of HPV73 at relapse.

Conclusion
This study showed that the inclusion of the evaluation of the HPV genotype specific persistence may represent a valid option to follow patients treated for CIN2+ lesions. In our study we found that relapses were detected only in patients with persistence of the same genotype detected at baseline.
HPV type specific distribution in women attending routine cervical screening in rural Malawi

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Background / Objectives

Population specific HPV prevalence studies are useful for planning vaccine and screening strategies. Our objective was to assess the prevalence of high-risk (HR) and low-risk (LR) HPV in women attending routine cervical screening in rural Malawi using a new, analytically sensitive genotyping assay.

Methods

Provider-taken and self-taken specimens were obtained between January 2016 and March 2017 from women attending routine VIA (Visual Inspection with Acetic acid) clinics in Nkhoma Hospital and associated Health Centres. All samples had previously been tested using the Xpert® HPV assay. Samples were classified based on VIA and Xpert results into four categories: VIA+/Xpert+; VIA+/ Xpert -; VIA- Xpert +; VIA- Xpert -.

A multiplex real-time PCR based assay (Papilloplex Any HPV; GeneFirst, UK) was performed which provides individual genotyping in two tubes (14 HR-HPV:16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 56, 66, 68(a&b) and 16 LR-HPV: 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 67, 69, 70, 72, 81, 82). HPV type specific prevalence of HPV in all four categories and concordance with Xpert were assessed.

Results

High concordance was seen in HR-HPV positivity between Xpert and Papilloplex HPV in provider-taken {proportional agreement=0.98 (95% CI- 0.79-0.90), k=0.68 (95% CI- 0.58-0.79)} and in self-taken samples {proportional agreement=0.97 (95% CI- 0.77-0.92), kappa=0.72 (95% CI- 0.57-0.80)}.

HR- and LR-HPV prevalence is summarised in Table 1. HPV 16 was most common in VIA+ women (N=156) followed by 52, 18, 51, 35, 31/33/45. HPV 6/44 then 67/72 were the most common LR-HPV. HPV 16 was also most common in VIA- women (N=139) followed by 52, 51, 35, 58 and 31/33 with the most frequent LR-HPV being 6 then 44/67/72/54/61. In women with a VIA assessment of ‘suspicious/frank cancer’ (N=42), HPV 16 was most frequent followed by 18, 45, 52, 31 and 51.
## Conclusion

In both VIA+ and VIA- groups, HPV 16 is the most common HPV type in women attending screening clinics in rural Malawi followed by types 52, 51, 35, 18 and 31. However, HPV 16, 18, 45 are the three most common types found in visually assessed suspicious cancers. High concordance was seen between the two tests but analytically sensitive Papilloplex assay detects higher prevalence of HR HPV prevalence. Further studies on type specific prevalence using analytically sensitive assays are warranted in clinical settings in low income countries.
BURDEN OF CERVICAL HPV INFECTION AND GENOTYPE DISTRIBUTION AMONG WOMEN ATTENDING TWO RURAL HEALTH CENTERS IN THE GONDAR REGION OF ETHIOPIA


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Background / Objectives

Cervical cancer prevention through primary vaccination is a promising approach especially for countries with high cervical cancer prevalence. Geographical differences of HPV genotype distribution are known, however little data are available for Ethiopia, the second most populated country in Africa.

Methods

Women were recruited as part of a visual inspection with acetic acid (VIA)-based cervical cancer screening program in 2 health centers in the Gondar region of Ethiopia. All consenting women underwent VIA preceded by collection of a cervical specimen for HPV testing using the Hybrid Capture 2 (HC2) assay (Qiagen™). HPV-DNA-positive samples were genotyped applying a bead-based hybridization assay using LumineX technology. All women with abnormal findings were referred to gynecologist for further management.

Results

700 women aged 18-64 years (median 35, IQR 27,40) were enrolled in the study of which 73 (10.4%) were HPV positive.

The most common high risk HPV genotypes were HPV 16 (55.6%), HPV 53* (22.2%), HPV 56* (13.3%), HPV 52 (11.1%), HPV 31 (8.9%), HPV 39* (6.7%), HPV 58 (6.7%), HPV 18 (4.4%), HPV 35* (4.4%), HPV 70* (4.4%) [*not included in nonavalent HPV vaccine against types 6, 11, 16, 18, 31, 33, 45, 52, and 58].

Conclusion

These data inform on the discussion regarding use of second versus first generation HPV vaccine for the country. More data are needed regarding HPV genotype distribution and correlation to disease status in Ethiopia.
P11-07
Genotyping of human papillomavirus in triaging of low-grade cervical cytology

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Background / Objectives

Atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intra-epithelial lesions (LSIL) are minor lesions of the cervical epithelium, detectable by cytological examination of cells collected from the surface of the cervix of a woman. Usually, women with ASCUS and LSIL do not have cervical (pre-) cancer, however a substantial proportion of them do have underlying high-grade cervical intra-epithelial neoplasia (CIN, grade 2 or 3) and so are at increased risk for developing cervical cancer. Therefore, accurate triage of women with ASCUS or LSIL is required to identify those who need further management.

The objective of the study was to evaluate whether typing of human papillomavirus among women with low-grade cervical cytology can improve the ability to identify women with cancer or cervical intrepithelial grade II+ (CIN II or worse).

Methods

This is prospective observational study carried out in Germans Trias i Pujol Hospital in Barcelona. A total of 266 women with low-grade cervical cytology participating in the study.

We used residual liquid-based cytology samples for HPV genotyping. Extracted DNA was subjected to parallel polymerase chain reactions using three primer sets for HPV DNA amplification. HPV+ samples were genotyped by DNA sequencing.

During 24 months, we study persistence and evolution of LSIL and ASCUS by citology and colposcopy each 6 months and HPV genotyping each year.

We study the individual and combined risk of progression depending on each HPV

Results

The adjusted prevalence of cervical intraepithelial neoplasia grade 2 or greater in our study was 23.5%.

The odds of persistence and progression were higher in women infected with HPV 16, 18 and 31.
HPV 16 was detected in 40% of cases with CIN II or worse but only among 24% of all tested women. HPV 31 was detected in 20% of cases with CIN II or worse but only among 11% of all tested women. Testing the three HPV types with higher risk (HPV16/18/31) detected 71% of CIN II or worse, with 36.9% testing positive. Positivity of other high risks HPV types had decreased risk of CIN III.

Conclusion

HPV genotyping may aid in prognosis of LSIL course. We should include HPV 31 in triaging LSIL, as it is the second most frequent HPV type involved in progression.

Different high-risk HPV types confer different risks for the presence of CIN2 or worse, implying that genotyping could be a useful optimization of triaging strategies.

References


Background / Objectives

Cervical cancer is the second leading type of neoplasia in Mexican women and high risk HPV has been associated with its development[1, 2]. Variations in HPV genomes have been associated with the severity of cervical lesions [3, 4]. We determined genetic variations founded in L1 region from different HPV types isolated from northeastern Mexican patients and compared them with the results obtained with real time PCR genotyping kit.

Methods

We collected 255 cervical samples from patients who attended colposcopy consultation at the Hospital Universitario “Dr. Jose Eleuterio Gonzalez” of the Universidad Autónoma de Nuevo León in Monterrey, Nuevo Leon, Mexico. One hundred forty-one samples were HPV positive, and genotyped using the E. coli Amplisens HPV HRC Genotype titre FRT kit using the AB7500 Fast Real Time PCR. We detected 43 HPV monoinfected samples and amplified the L1 region using PGMY 09/11 primers and sequenced the PCR product. The obtained sequences were assembled, and posteriorly analyzed with MEGA7. We built a phylogenetic tree with the maximum likelihood method using the GTR +G model [4, 5].

Results

The most frequent HPV was HPV 52. Seventy percent of our samples were infected with more than one HPV type. We identified 43 HPV strains from Mexican patients, were the 14% of those patients had a persistent HPV infection. Most of the patients (41%) presented a clinically valuable viral load. These strains had different genetic variations in the L1 region, and most of them were synonymous. There was no significant association of the detected HPV type with the viral load. Twenty HPV sequences differed from the HPV detected by real-time PCR.
Conclusion

The discrepancy between the HPV type detected by real-time PCR and Sanger sequencing was high [6]. This could be due to coinfections with several HPV types, with higher viral loads. We speculate that the included HPV probes cross-react with other HPVs not included in the kit [7]. Due to the samples background and the high frequency of the HPV coinfections in our population, there is a need to isolate HPV strains to evaluate their carcinogenic potential and the genetic evolution of these viruses circulating in the northeastern region. Even if their viral load was not associated with the HPV type, the genetic variations found could explain the carcinogenic potential these strains might have.

References


P12-01  
DIAGNOSTIC VALUE OF HPV16 AND HPV18 VIRAL LOAD AND INTEGRATION STATUS AMONG AFRICAN WOMEN INFECTED WITH HIV

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Background / Objectives

To evaluate the performance of E6 HPV16/18 Viral load, (E6-E2) HPV16/18 Viral load (viral load "integrated") and E2/E6 ratio (integration coefficient to detect cervical high-grade lesions (CIN2 +)) in a cohort of African women infected with HIV enrolled in the HARP (HPV Research Partnership in Africa) project. A total of 1238 women were enrolled in the HARP study. At baseline and at 18 months of follow-up (endline) the following screening tests were performed: HPV test, Cytology, VIA / VILI, colposcopy and biopsy if one test positive. Histology was systematically confirmed by the End Point Committee. In addition, HPV detection and genotyping was performed using the INNO-LiPA HPV genotyping Extra assay. 245 women co-infected with HPV16 and/or HPV18 were included in the present study. Their median (range) age was 35 (25-49) years, CD4 + cell count was 417 (7-830) cells/µL, and 158 (66.4%) women were on ART; 122 positive for HPV 16 and 78 for HPV18 at inclusion; 103 positive for HPV 16 and 66 for HPV18 at 18 months. 25 co-infected at baseline and 11 at endline.

Methods

Quantitative real-time PCR targeting the E6 and E2 genes were performed using serial dilutions of HPV16 and HPV18 plasmids as standard curves. Total cellular DNA was measured by real-time PCR of the GAPDH gene, and the results were expressed as the number of copies of E6 and E2 per 1,000 cells. SiHa, Caski and Hela cell lines were used as controls.

Results

It was observed a very significant (p<0.0001) increase in HPV16 E6 viral load, and HPV16 (E6-E2) viral load and a decrease in E2/E6 ratio as the grade of cervical lesions increased. It was also observed an increase (p=0.029) in viral loads with the lesion grade. There was a strong correlation between viral loads E6 and E2-E6 HPV16 and HPV18 (Rs = 0.899 and 0.962 respectively). E6-E2 and E2/E6 are not independent of E6. E6 viral loads of 4 log copies/1,000 cells for HPV16 and of 2 log copies/1,000 cells were associated with high grade lesions. Sensitivity and specificity
of these viral load levels were 83% and 75% for HPV16, and 50% and 70% for HPV18, respectively.

**Conclusion**

A high HPV16 viral load (> 4 log E6 DNA copies / 1000 cells) or, to a lesser extent, for HPV18 (> 2 log E6 DNA copies / 1000 cells) is associated with cervical high-grade lesions. Among women without high-grade lesion at baseline, a high HPV16 viral load is associated with progression to high-grade lesion at 18 months, this was not observed for HPV18. E6 Viral load and E2-E2 ratio are not independent markers of viral load E6. HPV16 viral load might constitute a triage test for HPV16-positive women. HPV18 viral load is of more limited interest given the low sensitivity / specificity ratio.
THE USE OF P16/KI-67 DUAL STAINING TECHNOLOGY ON CERVICAL CYTOLOGY OF PATIENTS UNDERGOING A LLETZ PROCEDURE

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Background / Objectives

The main objective of this prospective observational study was to investigate the diagnostic performance of the p16/Ki-67 DST for detecting CIN 2+ in comparison with HR-HPV testing and Pap cytology in a LLETZ referral population. Secondary study objectives were investigation of the diagnostic performance of the DST for triage of patients with persistent low-grade CIN (ASCUS or LSIL cytology results during > 24 months) or an inconclusive colposcopy examination.

Methods

A total of 110 patients referred for a LLETZ were enrolled between October 2016 and mid-March 2017. From each participant, a cervical cytology sample was obtained before the onset of the LLETZ procedure. On each sample, we conducted the DST (Roche CINtec Plus Test®), Pap cytology and an HPV DNA assay (identifying 17 different HPV types, including the 13 “high-risk” genotypes). Test results were correlated with the cone biopsy result to guarantee excellent disease ascertainment.

Results

The overall disease prevalence of CIN 2+ was 56%. The mean age was 41 years, with 38% of patients being younger than 35 years. P16/Ki-67 positivity increased with histological severity. Positivity was 35% in CIN 0, 46.6% in CIN 1 and 80% in CIN 2 patients. Positivity increased to 95.9% and 100% in cases of a histological diagnosis of CIN 3 or invasive carcinoma. The overall sensitivity and specificity of the DST for detecting CIN 2+ was 94% and 58% respectively with a PPV of 74% and a NPV of 88%. HR-HPV testing results in a similar sensitivity of 92% but considerable lower specificity of 21% compared to the DST. When ASCUS or worse is considered a positive test result, Pap cytology still has the lowest sensitivity of 89% compared to dual staining and HR-HPV testing. In cases of persistent low-grade CIN (n=19), the DST had a non-inferior sensitivity of 100% and superior specificity of 67% for detecting CIN 2+ compared to HR-HPV testing. In cases of an inconclusive colposcopy examination, the DST provides a sensitivity of 95% and negative predictive value of 94% for detecting or excluding relevant disease.

Conclusion
The p16/Ki-67 DST provides high sensitivity and improved specificity compared to HR-HPV testing and Pap cytology for predicting CIN 2+, making it an interesting tool for identifying relevant disease in patients referred for a LLETZ. Test performances were even better in patients referred with persistent low-grade CIN, but conclusions should be drawn with care because of the low number of patients in this subgroup (n=19). In cases of an inconclusive colposcopy examination, the DST seems to provide an excellent negative predictive value for excluding almost all relevant disease.
ANALYSIS OF THE INFLUENCE OF P16 IN THE INTER AND INTRA OBSERVER CONCORDANCE IN THE DIAGNOSIS OF INTRAEPITHELIAL NEOPLASIA OF CERVIX GRADE 2 (CIN2)

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Background / Objectives

The histological classification of the pre-neoplastic lesions of the cervix contemplates three categories, cervical intraepithelial neoplasia (CIN) grades 1, 2 and 3. This classification is based on a subjective assessment of the thickness of the affected epithelium: CIN1 is considered the affectation of a third or less, while the thickness of the affected epithelium in CIN3 affects more than two-thirds of its thickness. It is established that the really pre-cancerous lesion is the CIN3. But between CIN1 and CIN3 we have the CIN2, an equivocal diagnosis, given the established very low inter-observer agreement to establish its diagnosis, about 30% according to data available in the literature.

We have today a biomarker, the p16, a marker of viral integration, which can objectify the diagnosis of CIN2 and, consequently, improve its concordance and diagnostic safety.

To assess inter and intra-observer agreement in the diagnosis of CIN 1 - 2 and 3, and to study how the use of the p16 modifies these values.

Methods

We collected 100 biopsies of cervix diagnosed with CIN2 from our records between 1997 and 2007. We performed p16 in all of them.

In a first phase, three expert pathologists evaluated 297 cervix biopsies, including cases of CIN2 randomly inserted along with cases of CIN 1, CIN 3 and invasive carcinoma of the cervix. They have subsequently analyzed p16 separately. In a third phase, the CIN2 biopsies have been passed along with their corresponding p16 for assessment and diagnosis. To conclude, 150 of the 297 biopsies were randomly selected to evaluate inter-observer agreement.

Results

The final results of the work will be presented at the Congress.

Conclusion
The final results of the work will be presented at the Congress.

References


P12-04
PROTEOMIC COMPOSITION OF CERVICOVAGINAL FLUID IN HPV-ASSOCIATED CERVICAL LESIONS

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Background / Objectives

To determine changes of the cervicovaginal fluid (CVF) proteomic composition for assessment of the severity of HPV-associated cervical lesions among reproductive age women.

Methods

The study involved 30 women with various forms of HPV-associated cervical lesions (ASCUS, LSIL and HSIL). All samples of cervicovaginal fluid were prepared for further proteomic analysis by tandem mass spectrometry (HPLC-MS/MS). Semi-quantitative data analysis including identification and annotation of proteins was carried out using the software package MaxQuant and Perseus.
Results

The protein panels specific to the various forms of HPV-associated cervical lesions (ASCUS, LSIL and HSIL) were identified. The first group of proteins (P4HB, HSPA8, C4BPA and others) characterized the early changes associated with HPV infection and cervical epithelium lesion, including penetration of viruses into the cell and its transcription, impaired function of the complement system. The second group of proteins (PRDX5, YWHAE, LRG1 and others) were directly involved in the development and progression of cervical neoplasia and characterized late changes, in particular, reduced apoptosis, impaired differentiation and maturation of the epithelium, and the transformation of atypical cells.

Conclusion

The protein composition of the CVF was studied to assess the severity of HPV-associated cervical epithelial lesions in reproductive age patients by tandem chromato-mass spectrometry (HPLC-MS / MS). The protein panels, specific for various forms of HPV-associated cervical epithelial lesions are determined (the first group - P4HB, HSPA8, C4BPA, the second group - PRDX5, YWHAE, LRG1).
DETECTION OF CERVICAL (PRE)CANCER ON THE BASIS OF CERVICOVAGINAL FLUID: POSSIBILITIES FOR DEVELOPMENT OF A SELFFTEST

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Background / Objectives

Despite tremendous efforts over the last decades, current screening methods for cervical cancer still have limitations in sensitivity and/or specificity. Moreover, vaccines are not effective against all HPV types and efficiency is uncertain in case of previous infection. In the search for more specific and sensitive biomarkers, and additional challenge represents the application of these biomarkers in low- and middle income countries where the incidence of cervical cancer is highest. The Cervico Vaginal 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 self-sampling as a good and acceptable sample collection method for subsequent DNA genotyping, cytology or immunohistochemistry, CVF may very well be suited for the development of a selftest for triage of suspected cases or screening in low- and middle income countries.

Methods

A differential proteomics study on CVF was performed using six CVF samples from healthy and six samples from precancerous women. Extracted proteins were run over a 2D-LC-MS/MS platform and quantified by spectral counting. Lists of identified CVF proteins were analyzed by Ingenuity pathway Analysis (IPA) to find out whether cervix cancer pathways were reflected in the CVF. A series of candidate biomarker proteins was further validated by ELISA or mass spectrometry (MRM).

Results

We identified alpha-actinin-4 (ACTN4) as a protein biomarker that could discriminate between the healthy and (pre)cancerous states with a sensitivity and specificity of resp. 84 and 86%. Based on the list of proteins that were differentially abundant in both types of CVF, a set of cervical cancer protein biomarkers interconnected within several cancer-related pathways was identified by Ingenuity Pathway Analysis (IPA). We quantified these biomarkers by ELISA or mass spectrometry (MRM) in CVF samples from healthy or precancerous woman in order to further increase the discriminative power in combination with ACTN4.
Conclusion

The cervical vaginal fluid may contain several biomarkers which, when used in an appropriate combination, could be used for development of an accurate cervical cancer screening test. Since collection of CVF is non-invasive, these biomarkers allow for the development of a self-diagnosis test to be used for screening, prediction or follow-up of cervix cancer.

References

CLINICAL UTILITY OF p16INK4a AS A DIAGNOSTIC ADJUNCT FOR UNDERLYING CIN2+ CERVICAL LESIONS

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Background / Objectives

In Honduras, premalignant and malignant lesions of the cervix continues to represent a major burden on the health care system mainly due to decreased specificity of screening tests as well as significant interobserver variability in the diagnosis of these lesions. Since p16INK4a is a surrogate marker of HPV E7-mediated pRb catabolism, it has been successfully deployed for the classification of HPV-related disease. This study aimed to assess the clinical significance of overexpression of p16INK4a in cervical lesions.

Methods

To help delineate the utility of p16INK4a, colposcopy-directed biopsy samples drawn from a larger study (n = 20: negative, 9; CIN I, 3; CIN II, 8) were analyzed by immunohistochemistry for expression of p16INK4a. Testing for high-risk human papillomavirus types by Hybrid Capture2 and genotyping by L1 HPV region PCR (GP5/6+) followed by reverse hybridization (LiPA) was performed on concurrent cervical scrape specimens.

Results

None of the negative and CIN I cases (n=12) expressed the p16INK4a protein. On the other hand, all CIN II specimens (n=8) were positively associated with p16INK4a expression and high-risk HPV presence (P < .001), showing a sensitivity and specificity of 100% (95% CI: 75.7-100.0). The HPV prevalence in the negative and CIN I cases was 50% as opposed to 100% of CIN II cases. The viral types identified in the CIN II cases were 16, 18, 35, 58, 51 and 66, being HPV16 the most common.

Conclusion

Although a small sample size, our findings show a possible utility for adjunct p16INK4a in addition to HR-HPV testing to distinguish between negative/low-grade (CIN 1) and high-grade squamous intraepithelial lesions (CIN II+) to avoid overtreatment of false-positive cases and under treatment of false-negative cases. It is suggested to test a larger sample size to increase the statistical significance of the study.
MODERN MULTIDISCIPLINARY MONITORING OF CERVICAL CANCER RISK

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Background / Objectives

Currently, Romania is ranked first in Europe in terms of cervical cancer mortality. In this context, the solution which has been developed in recent years is the secondary use of molecular markers more specific for cervical precancer, combining their high sensitivity with high specificity. Among these methods, p16 / Ki67 dual immunocytochemistry is the most studied.

Methods

One hundred and eighty-three patients who performed the Papanicolaou test, the HPV-DNA test and the immunocytochemistry test (CINtec PLUS) from June 2014 to June 2017 were examined. Patients with the positive CINtec PLUS test were recommended for a colposcopy examination and subsequent biopsy.

Results

The sensitivity and the negative predictive value of CINtec PLUS was 100%, the specificity 75.2% and the positive predictive value 60.2%. Performing a double staining test in patients with ASCUS type cervical cytology changes in the study group has been shown to be very effective in identifying precancerous cervical lesions.

Conclusion

Thus, by using the p16/ Ki-67 immunocytochemical staining, the medical attitude in screening or monitoring young patients with HPV infection, high-risk strain and ASC-US, the results being optimized. Unnecessary colposcopy examinations are avoided (indications of colposcopy are restricted to CINtec PLUS Positive and invasive gestures on nulliparous women are limited. CINtec PLUS negative results are monitored by repeating cytology testing and HPV DNA testing at 12 months.
Background / Objectives

Most cervical cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV). Genetic and epigenetic changes as the silencing of inhibitors WNT signaling pathway can affect the outcome of HR-HPV infection. Considering that the methylation of DNA is important for the carcinogenic process, the aim of this study was to analyze status of methylation of DKK3, SOX17 and SFRP2 genes regarding HR-HPV types 16/18/45, staging, degree of differentiation and origin of cervical cancer.

Methods

A total of 169 paraffin-embedded tissue blocks from biopsies performed in cervical cancer patients were selected. HPV detection and genotyping were performed using the INNO-LiPA HPV Genotyping assay. After treatment with sodium bisulfite, the samples were submitted to MS-PCR

Results

The age of the patients at the time of diagnosis of cervical cancer ranged from 26 to 91 years, with an average of 52.3 years (95% CI = 49.3-54.7). The mean age of the patients who were diagnosed with adenocarcinoma was 46 years (95% CI = 40.7-51.3). Squamous cell carcinoma on average affected 53.9 year old women (95% CI = 49.7-56.2). A total prevalence of HPV was 94.3%. All cases as diagnosed squamous cell carcinoma were positive for HPV among cases of adenocarcinomas, 86.4% were positive for HPV. The methylation of genes was a prevalent event in cervical cancer ranging from 65.5% to 90.0%. The prevalence of HPV 16, 18 and 45 was 82.2%. Infection with HR-HPV showed a significant association for SFRP2 (p=0.05).
Methylation of the SOX17 gene was positively associated with lower severity of stages of cervical cancer (p=0.04). The methylation of the SOX17 gene was associated with the presence of well or moderately differentiated tumors (p=0.01). When all genes were considered an association with better differentiation was observed (p=.0.05) In addition, there was a significant association between infection by the HPV 16, 18 and 45 and the diagnosis of adenocarcinoma (p=0.01). A borderline association was observed between the methylation of the DKK3 and SOX17 genes and the diagnosis of adenocarcinoma (p=0.07).

Conclusion

The methylation of inhibitors WNT signaling pathway and HPV 16, 18 and 45 infections are frequent event during multistep carcinogenesis, however, only was significant association with SFRP2 metilation. SOX17 metilation was be related with lower cervical cancer severity but not with HPV types. Adenocarcinomas showed a significant association with HPV 16, 18 and 45 infections, however showed a borderline association with DKK3 and SOX17 metilation.
AN MRNA PANEL FOR TRIAGE OF HPV POSITIVE WOMEN WITH HIGH SPECIFICITY FOR DETECTION OF CLINICALLY RELEVANT CERVICAL DISEASE

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Background / Objectives

HR-HPV based primary cervical screening modalities are now being implemented in several countries. Robust objective triage strategies for management of HR-HPV positive women are needed. The objective of this study was to identify mRNA levels of chemokines in liquid based cytology samples for use as biomarkers for the risk stratification of HR-HPV positive women.

Methods

A panel of cervical liquid based cytology samples derived from both screening and colposcopy populations were tested for HR-HPV using the rT-HPV Test (Abbott, USA) and mRNA expression levels of eight chemokines CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL8, CXCL10 and CXCL12 through singleplex TaqMan RT-PCR. A case-control analysis comparing samples from HR-HPV positive women with CIN2+ (n=48) to women with no disease (defined as normal colposcopy or histology <= CIN1, n=80) was performed with ROC curve analysis in order to determine initial clinical performance of the chemokine markers.

Results

Significant differences (p≤0.05) were seen in expression of CCL2 and CCL5 between women with and without significant disease. AUC of CCL2 is 0.61 (95% CI- 0.51- 0.71) and CCL5 is 0.60 (95% CI– 0.49- 0.71).

Conclusion

Our initial data show that assessment of chemokine mRNA levels for the detection of HPV associated significant disease has promise. Future work will be aimed at further development and optimisation including the generation of a multiplex real-time PCR which will incorporate the most informative targets.
WHAT IS THE POSITIVE PREDICTIVE VALUE OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL) ON CYTOLOGY FOR THE HISTOLOGICAL DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2 (CIN2) OR MORE? A SYSTEMATIC REVIEW

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Background / Objectives

As cervical cancer is a major health problem, regular cervical screening to make an early diagnosis can help prevent cervical cancer, through identifying and treating pre-invasive cervical lesions. The aim of this review is to evaluate the correlation between the cytological screening and histological outcome in the diagnosis of cervical cancer, more specifically the correlation between HSIL on cytology and histological CIN2+. Learning if cytology brings up information about the probability to discover a high grade cervical intraepithelial neoplasia, would imply that the cytological screening program is a valuable tool on its own.

Methods

An electronic search was carried out in Medline (through Pubmed) and Cochrane (last searched in November 2016), supplemented with the related article feature in Pubmed and snowballing. Article selection (predefined inclusion and exclusion criteria), data extraction and methodological quality assessment (QUADAS) were evaluated by two independent reviewers.

Results

After identifying 1065 articles, 24 articles were included in this systematic review, representing 51,962 cytological HSIL women in total. The mean CIN2+ percentage in cytological HSIL women is 65,1% (range: 45,4% – 95,2%). The mean CIN3+ percentage in cytological HSIL women is 43,9% (range: 36,4% – 62,1%).

Conclusion

In this systematic review, the mean CIN2+ percentage in cytological HSIL women is 65,1%. The correlation between HSIL on cytology and histological CIN2+ is therefore fair but a biopsy is necessary to confirm high-grade disease.
Comparison of HPV positivity of vaginal samples harvested by gynecologist and patient herself in Japan

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Graduate School of Medical Science (Japan), 2Japanese Foundation for Sexual Health Medicine (Japan)

Background / Objectives

It seems to be difficult for young Japanese girls to seek gynecological clinic and mount on the examining table because of embarrassment. In order to increase rate of cervical cancer examining test in young girls, it may reasonable to adopt HPV self-harvesting test (S sample). However, before adopting self-harvesting test, it is necessary to verify the superiority or non-inferiority of that compared to harvesting by a gynecologist (G sample). In this report, the superiority of self-harvesting test was examined.

Methods

Seventy four patients having more than slight dysplasia of the cervical region were examined in this study by using paired samples taken by a gynecologist and a patient herself in the same visiting date. Samples were examined by using a kit of GENOSEARCH HPV 31 which can identify 31 HPV genotypes including 13 high risk and 18 low risk HPV types.

Results

In a result, HPV positive rate was higher but not significant in S samples of 78.4% (58/74 cases) compared to that in G samples of 70.3% (52/74 cases). Superinfection of HPV was likely to be detected more often in S samples than G samples. In each type of high risk HPV, positive case number of S samples was superior to that of G samples in most HPV types (7 vs 1). Quite same was observed in the case of low risk HPV types.

Conclusion

In summary, the HPV positivity of S samples was superior to that of G samples. It is considered that HPV sample-harvesting by patient herself is very useful not only for early diagnosis but also for early treatment of cervical cancer.

References

DISCORDANT RESULTS BETWEEN ONCOGENIC HUMAN PAPILLOMAVIRUS RNA AND DNA TESTS IN A COTESTING CERVICAL CANCER SCREENING PROGRAM.

A. Sáez 1, I. Gallego 2, P. Cano 3, I. Romero 3, T. Nieto 3


Background / Objectives

The aim of this study is to evaluate the discordant results between Cobas 4800 HPV test and E6/E7 mRNA Aptima HPV Assay.

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 the discordant results between them, were analyzed by Linear Array HPV Genotyping test (Roche Molecular System Inc)

In each patient we made Pap smears, and biopsy and p16 when the patient required it.

Statistics analyses was 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

<table>
<thead>
<tr>
<th>HPV</th>
<th>DNA Cobas 4800</th>
<th>mRNA AHPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>50.1% (369)</td>
<td>41.0% (302)</td>
</tr>
<tr>
<td>Negative</td>
<td>49.9% (367)</td>
<td>59.0% (434)</td>
</tr>
</tbody>
</table>
Kappa value DNA Cobas 4800 = 0.834 ; Kappa value mRNA AHPV = 0.805

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
Cobas 4800 DNA  sensitivity = 0.94 [95% CI: 0.91-0.96]  specificity = 0.90

Our Pap smear distribution, and the frequency of HPV in each category is shown in Table 2

<table>
<thead>
<tr>
<th>Cytology</th>
<th>% Cobas 4800 Positive</th>
<th>% APTIMA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=450)</td>
<td>28.4</td>
<td>20.4</td>
</tr>
<tr>
<td>ASCUS (n=56)</td>
<td>75.0</td>
<td>62.5</td>
</tr>
<tr>
<td>LSIL (n=108)</td>
<td>85.1</td>
<td>74.1</td>
</tr>
<tr>
<td>HSIL (n=112)</td>
<td>91.9</td>
<td>84.0</td>
</tr>
</tbody>
</table>

10 samples were AHPV positive/Cobas 4800 Negative. Eight of them were negative in the Linear Array Genotyping test. Regarding the two remaining samples, one was positive for HPVs 42+51 and the other was positive for HPVs 16+35+42+51

74 samples were AHPV negative/Cobas 4800 positive:

- 2 cases out of 74 were negative in Linear Array Genotyping test.

- In the remaining 72 cases: HPV 16 was present in 27% of these cases, HPV 18: 1.7%, HPV 31: 5.4%, HPV 33: 2.7%. All the remaining samples were positives for other HPV genotypes.

50% of them were coinfected with two or more viruses.

Conclusion

According to our data, the sensitivity of Cobas 4800 HPV test was higher (p=0.032).

Almost 30% of the samples with discordant result, E6/E7 mRNA-based Aptima HPV negative and Cobas 4800 HPV positive, had HPV 16/18.

In the screening programs of general population, we need to get more data, as E6/E7 mRNA-based Aptima HPV we are going to loose women with high risk HPV 16/18.
UPGRADING OF INFORMATION SYSTEM FOR MANAGEMENT AND MONITORING OF SLOVENIAN CERVICAL CANCER SCREENING PROGRAMME

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Background / Objectives

Due to rapid technology development and research, new evidence that accumulates and will continue to accumulate, enables risk-stratified screening and management of women participating in organised screening programmes. However, it is highly impractical for clinicians to integrate all this knowledge into classical, diagram based clinical algorithms that have traditionally guided clinical decisions. With the aim to overcome the complexity of clinical decisions we have decided to develop a concept of an innovative information system that would in the future enable cervical cancer screening and management of screen-positive women according to their risk for high-grade cervical lesions and cervical cancer within the organised, population based cervical cancer screening program in Slovenia.

Methods

Literature review was done to identify the most relevant information for stratifying individual risk for high-grade cervical lesion in such a way that screening, diagnostic and follow-up algorithms could be adjusted. Innovative IT supported solutions for clinical management decisions and guidance were identified in collaboration with IT experts.

Results

The following risk-stratifying factors were considered for the screening and management: women’s age, HPV-vaccination status, screening history, screening and follow-up tests and their results, colposcopy and histology report. The concept of upgraded central cervical cancer screening information system was developed with the objective that this information will be available to the professionals involved in screening and management of the women, together with the guidance tool for the clinical decisions based on current screening and management guidelines of Slovenian cervical cancer screening program. The system is based on structured, standardised reports and process platforms. New evidence can lead to a change in screening and management guidelines. Due to high information system flexibility the changes will be implemented only by parametrisation and configuration of the system without major changes in programming code.

Conclusion
Organised, population based screening programmes are entering the era where innovative technology solutions and new evidence from research are accumulating rapidly. This may change the traditional role of cancer screening registries from being used as an additional system within the screening programmes that allows for monitoring and evaluation of the programme, to the central communication and decision supporting tool between the professionals involved in screening, diagnostic, follow-up and treatment of women. Such active system also enables real-time monitoring and evaluation of the program.
Economic analysis of a strategy to improve cervical cancer screening in Denmark: Cytology with HPV triage vs. primary HPV screening with cytology and CINtec PLUS cytology triage

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Background / Objectives

Healthcare decision makers search for cervical cancer (CxCa) screening strategies that produce better clinical outcomes while controlling the cost. Possibilities for the introduction of primary HPV screening are currently investigated in Denmark. This modelling study compares clinical outcomes and costs of replacing; 1) cytology with pooled HPV triage (current practice), with 2) cobas® HPV with cytology and CINtec PLUS Cytology® triage (comparator) in the national CxCa screening programme in Denmark.

Sensitivity limitations and subjective interpretation of cytology may lead to missed diagnosis. The combination of primary HPV screening with cytology and CINtec PLUS cytology triage address this shortcoming. CINtec PLUS cytology confirms a transforming HPV infection by detecting cervical cells where HPV has disrupted cellular control (p16/Ki67+) and predicts which women most likely have precancerous cervical lesions and therefore benefit from an immediate colposcopy.

Methods

The model compares screening performance, clinical outcomes and costs. Screening of a hypothetical cohort of 796,000 Danish 30-59-year-old women and natural progression/regression of the disease are modelled for two screening cycles. In the current practice; women with normal cytology return to routine screening (30-49-year-old in 3 years and 50-59-year-old in 5 years), ASCUS and LSIL results have a reflex HPV triage, and HSILs undergo a colposcopy. In the comparator; HPV-negative women return to routine screening in 5 years. HPV+ are first triaged with cytology. Women with ASCUS or LSIL results have a reflex CINtec PLUS triage. Negative CINtec results and normal cytologies are followed up with an HPV retest and a reflex CINtec PLUS triage in two years. CINtec positives and HSIL cytologies undergo a colposcopy. Test sensitivity and specificity data are from ATHENA trial. Other inputs include the local prevalence of HPV, HPV genotypes 16/18, CIN1-3 and CxCa. All costs are calculated from healthcare provider’s perspective.

Results
The comparator strategy increases the sensitivity CIN2+ from the current 52.1% to 71.1% and maintains the specificity CIN2+ (current 99.1%, comparator 99.3%). The better screening performance reduces annual incidence of CxCa in the screened population from the current 23.0 to 18.9 per 100,000, and annual CxCa mortality from 5.1 to 4.1 per 100,000. The annual cost increase by 6%, from 79.2 to 83.8 million DKK, which is mainly caused by increasing treatment costs.

Conclusion

The results suggest that replacing the current practice, with primary HPV screening with cytology and CINtec PLUS triage produces better clinical outcomes and increases costs slightly.
ROLE OF HPV VIRAL LOADS IN GUIDING BIOPSY UNDER COLPOSCOPY FOR ASC-US AND HPV POSITIVE WOMEN

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Background / Objectives

Improvement remains on subsequent management of women with concurrent atypical squamous cells of undetermined significance (ASC-US) cytology and positive HPV results. The aim of our study was to explore the role of HPV viral loads in guiding biopsy under colposcopy for ASC-US and HPV positive women.

Methods

We performed a pooled analysis of 17 population-based cross-sectional studies conducted in China from 1999 to 2008. 30,371 women were screened with liquid-based cytology (LBC), HPV testing (hybrid capture 2, HC2) and visual inspection with acetic acid test (VIA) and diagnosed by colposcopically-directed biopsies. HPV viral loads were stratified as low [1.0, 10.0), intermediate [10.0, 100.0) and high [100.0, +∞) in RLU/CO value. Risks of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) among different viral load groups were analysed with linear trend Chi-square test and relative CIN2+ risks among groups were calculated with logistic regression analysis.

Results

908 ASC-US women with positive HPV and complete biopsy results were included in final analysis, among whom, 649, 170 and 89 women were diagnosed as normal, CIN1 and CIN2+, with median value of HPV viral loads as 23.15(4.46,121.91), 85.53(18.73,367.90) and 95.68(18.95, 370.34), respectively. CIN2+ risks increased significantly with elevating of viral load levels (p trend<0.001). Women with intermediate and high viral loads showed at least 67.60(20.52,222.50) times higher CIN2+ risk than ASC-US but HPV negative women. Among 37 CIN2+ cases missed by colposcopy, 72.9% were at intermediate to high viral load range and this proportion achieved 81.8% among cervical intraepithelial neoplasia grade 3 or worse(CIN3+) cases. As for ASC-US women at low viral load and relative lower CIN2+ risk, though an abnormal colposcopy result did not increase the CIN2+ risk
significantly, it showed 7.61(1.36, 42.62) times higher CIN3+ risk than a negative colposcopy result.

**Conclusion**

Intermediate to high HPV viral loads in ASC-US and HPV positive women effectively predict a significantly increased risk of existing CIN2+ and should be biopsied regardless of colposcopy results. As to those with low HPV viral loads, only abnormal colposcopy result are supposed to be referred to guide biopsy, ensuring high CIN2+ detection rate and less biopsy harms concurrently.

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Background / Objectives

To investigate the efficacy and efficiency of different screening programs along 3 periods of time:

2010-2013: Cytology (conventional or liquid based). HPV in ASCUS.


2016: Primary HRHPV testing. HPV parcial genotyping 16-18 and reflex testing

Methods

The base population in our area is 54,372 women. Trained midwives take screening samples in primary healthcare facilities.

In conventional practice, a referral for colposcopy is based on a cytology result: ASCUS and HPV(+), L-SIL, H-SIL, AGC, HPV 16-18 and negative cytologies at women aged > 30 years, and HPV (+) no 16-18 + 2 consecutive negative cytology results for 2 consecutive years.

HPV determination is performed with Roche cobas® 4800 HPV Test (COBAS).

We assessed CIN outcomes following reflex cytology and HPV genotyping for colposcopy triage.

Results

19893 cytologies (4.72 % pathological) were performed during the period of 2010-2013. The frequency of abnormal results was the following: ASCUS (3.63%), L-SIL (0.91%), AGC (0.025%) and H-SIL (0.15%).

For the 2014-2015 period, 10019 cytologies were performed (4.33% pathological), ASCUS (3.17%), ASC-H (0.08%), L-SIL (0.84%) and H-SIL (0.24%) respectively.
In 2016, 1,565 cytologies were performed (13.23% pathological) ASCUS (9.90%), ASC-H (0.45%), L-SIL (2, 43%) and H-SIL (0.45%)

Over the 2010-2013 periods, 2204 HPV determinations were performed, from which positive results were obtained in 27.08% of the cases. During 2014-2015, 8494 HPV determinations were reported, with 12.05% of the results being positive. 2758 studies were performed in 2016, and 10.88% were positive.

745 biopsies were performed during the period 2010-2016. Between 2010-2013, 233 biopsies were performed, (45.37% positive), 58 of them H-SIL. Over 2014-2015 314 biopsies were performed (50.48% positive), 108 H-SIL.

Lastly, in 2016, 198 biopsies were performed (52.33% positive), 69 H-SIL. If we compare the first and the last screening period, positive biopsies increased 241% and H-SIL diagnosis around 375%.

Conclusion

Comparing the first and the last screening period, cytologies have been reduced in a 68.5%, which means a medical cost saving. A big increment is shown in abnormal cytologies during the HPV primary screening.

Closer follow-up of clinical guidelines explains variations in HPV positivity

No benefits are reported from using co-test as first option for the screening program.

A significant increase in the number of colposcopy biopsies was observed over time, with a slight increase in positive biopsy result, but a better diagnosis of H-SIL.

To develop new screening strategy options with the goal of minimizing unnecessary follow-up visits.

References

THE IMPLEMENTATION OF HPV BASED SCREENING IN AUSTRALIA: SUSTAINABLE WORKFORCE IMPLICATIONS.

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Background / Objectives

In December 2017 the National Cervical Screening Programme (NCSP) in Australia will change from a 2 yearly conventional cytology based approach to 5 yearly HPV DNA testing. A positive partial genotyping test result for HPV 16/18 will go to reflex LBC and patient referral for colposcopy; detection of other oncogenic types will undergo reflex LBC triage. Among the myriad technical hurdles that must be cleared and human resource elements affected by this change will be a substantial reduction in the workload of cytology laboratories and the role of cytologists and pathologists. The paradigm shift in the primary screening platform has changed the role of the cytologist to a diagnostic one. Thus the experience of the cytologist in partnership with the pathologist is key to the success of the reflex and co testing follow-up investigations. It is estimated that following the changeover around 1 in 5 cytologists will be retained to service the predicted LBC workload. The immediate challenge of the reshaping period for laboratories that specialise in gynaecological cytology has been to manage the opposing forces of maintaining service efficiencies while their workforce is restructured. Specific planning for the preparation of future cytologists is a challenge facing pathology laboratories, tertiary education centres that train undergraduate scientists in diagnostic cytology and professional bodies responsible for continuing education, quality assurance and performance measures.

Methods

This study aims to encapsulate the available information around the introduction and management of HPV based screening in Australia in 2017 and strategies to deal with pathology workforce issues. Estimates of the changes to the national workload indicate that cytology tests will be considerably fewer (1). Available information around anticipated changes to work practices by major public and private pathology providers in relation to the workforce transition is presented. Strategies that facilitate future diagnostic cytology training at university level and in the workplace are tabled.

Conclusion

The theoretical endpoint for the NCSP in Australia is prevention of all HPV related anogenital carcinoma through vaccination and improved screening outcomes. The role of cytology in this pursuit is changing but will remain a key component for the foreseeable future. Appropriate training regimens for cytologists and pathologists that will ensure ongoing diagnostic acuity are essential for service provision.
References

IS A THREE-YEAR CYTOLOGICAL SCREENING FOR CERVICAL CANCER SAFE AND IS IT IMPORTANT FOR POSSIBLE EARLY TREATMENT OF PREMALIGNANT INTRAEPITHELIAL LESIONS?

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Background / Objectives

Screening for cervical cancer implies early diagnostics of high-risk intraepithelial lesions (HSIL) and the initial microinvasive changes. The method which is recognized as the gold standard for this screening is exfoliative cytology - PAP test. Recommendations for safe time interval that should repeat cytology screening are 3 years. In this interval, calculated risk of carcinoma is 0.8%. It is known that the sensitivity of conventional PAP test is from 30 - 85%. The influence of subjective factors and errors in collecting and interpreting the findings reduce the validity of the test. According to official recommendations, it is considered that the repetition of PAP test in the three-year interval is safe in screening of cervical carcinoma. The aim of this study was to determine the reliability of conventional PAP test in screening for cervical cancer and to determine what percentage of the HPV test can increase the reliability of PAP test in screening for cervical cancer.

Methods

The study entered 39 patients, which occurred in 2017, for surgical treatment of HSIL and invasive cancer (IC). In all patients, the PAP test was done and also HPV PCR test. A history of time interval since the last normal NILM findings as well as data on the possible contact or irregular bleeding, are also entered in the statistical analysis of data.

Results

Only 20,51% of patients had a PAP test done before three years or more. 35,8% of the patients had normal test result less than 3 years and 43,58% of them done the PAP test a year ago and the result was also normal - NILM. From the 20 patients with IC, 16 of them (80%) had normal PAP test less than 3 years. 40% (8 patients) had a normal PAP test a year ago. Analysis of the documents that they have been brought with them, as part of preoperative preparation, showed in 11 patients (28,2%) with HSIL and IC normal PAP findings. From these 11 false-negative cytological findings (FNF), in 4 (36,36%) has been diagnosed HSIL and in 7 (63,6%) IC. From these 7 with IC, in 4 patients with (57%) FNF, histological type was adenocarcinoma. A total sensitivity of cytology was 71.79%. HPV testing in 38 patients (97%) with HSIL and IC was positive. Only one patients with HSIL (2.56%) had a negative HPV test. HPV test increased sensitivity of cytology for 25.64%.
Conclusion

The three year cytological screening with conventional PAP test, as a alone test, is not reliable for screening of cervical cancer. HPV test, in combination with cytology, increases the overall sensitivity of cytology for 25.64%. Both of them are very important for early treatment of premalignant intraepithelial lesions.

References


P13-10
Compare Two Different Usages of the FRD™ for Detecting High Grade Cervical Lesions and Invasive Cancer

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Background / Objectives

To evaluate the two usages of the Folate Receptor-Mediated Staining Solution (FRD™) for detecting CIN2+, and compare it to TCT and HPV testing.

Methods

The FRD™ is a staining method for rapid visualization of CIN2+. Test results are determined immediately with the aid of the FRD™ Colorimeter after staining of the entire cervical epithelium. The two methods of the FRD™ test were performed on patients who had ASC-US & above TCT, and/or positive HPV test, before undergoing colposcopy and biopsy. The first FRD™ testing method (sampling method), the cervical epithelium is collected with the Epithelium Staining Applicator, and then the applicator is stained by the FRD™ staining solution. The second method (direct dyeing method), the Epithelium Staining Applicator is dipped into the FRD™ staining solution, and then the cervical epithelium is collected and stained by the applicator.

Results

317 women with histological findings were included. CIN2+ was found in 109 women (34.38%) including 16 cervical cancer cases (3.3%). CIN1 and negative cases accounted for 9.46% and 56.15%, respectively. TCT results included NILM in 103 women (32.49%), ASC-US in 130(41.01%), LSIL in 51(16.09%), ASC-H in 12(4.73%), and HSIL & above in 21(6.62%). HPV positive rate was 90.54% (287/317). Positive FRD™ test was determined in 35.33% women (112/317) by the sampling method, and 48.90% (155/317) by the direct dyeing method. The sensitivity to detect CIN2+ for abnormal TCT, positive HPV, and positive FRD™ by the sampling method and direct dyeing method were 69.72%, 97.25%, 64.22%, and 81.65%, respectively. The specificity to detect CIN2+ for abnormal TCT, positive HPV, and positive FRD™ by the sampling and direct dyeing method were 37.98%, 12.98%, 78.81%, and 68.27%, respectively.

Conclusion

Compared with TCT and HPV test, both the usages of the FRD™ had a compatible sensitivity and high specificity to detect high grade cervical lesions. The sensitivity of the direct dyeing method was higher than the sampling method, and its specificity was lower than sampling method, but there was no significant difference between them. Sensitivity is more significant in cervical cancer detection, therefore the direct dyeing method of the FRD™ is more suitable in clinical settings. In addition, the FRD™ is a very inexpensive and easy method, which can be used in less-developed
counties or areas that lack the resources and trained personnel required for routine cervical cancer detection.
The Significance of the Epithelium Staining Applicator in Cervical Staining with the FRD™

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Background / Objectives

This study was aimed to evaluate the significance of the Epithelium Staining Applicator in cervical staining with the Folate Receptor-Mediated Staining Solution (FRD™) in detecting cervical abnormal lesions (CIN2+), based on biopsy being used as the gold standard.

Methods

The FRD™ test was performed before colposcopy, on patients with abnormal TCT and/or positive HPV test. The cervical epithelium was stained by the Epithelium Staining Applicator, by first dipping the applicator into the FRD™ staining solution, and then by pressing the applicator against the cervix with the aid of speculum. After staining, the Epithelium Staining Applicator was placed into the FRD™ Colorimeter, which scans the applicator for any color change and prints out the scanning results. The FRD™ test results were determined by the readings found on the scanning results.

Results

261 women with histological findings were included in the study. CIN2+ was found in 97 patients (37.16%) including 12 cervical cancers cases (4.60%). CIN1 accounted for 10.34%. TCT results included NILM in 82 women (31.42%), ASC-US in 114 women (43.68%), LSIL in 42 women (16.09%), ASC-H in 7 women (2.68%), HSIL and above in 16 women (6.13%). The HPV positive rate was 87.36% (228/261). A positive FRD™ test was determined in 49.81% women (130/261). The sensitivity to detect CIN2+ lesions for abnormal TCT, positive HPV, and positive FRD™ were 76.29%, 93.81%, and 80.41%, respectively. The specificity to detect CIN2+ lesions for abnormal TCT, positive HPV, and positive FRD™ were 35.98%, 16.46%, and 68.29%, respectively.

Conclusion

The FRD™ is an alternative method which is suitable for cervical cancer detection. In addition, the Epithelium Staining Applicator is a suitable assistive device to complete the FRD™ test.
The diagnostic value of lugol solution, acetic acid, and Pap smear compared to biopsy regarding premalignant and malignant cervical lesions diagnosis in patients in need of colposcopy

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Background / Objectives

Given the high incidence of cervical cancer in developing countries and the importance of prompt diagnosis and treatment regarding mortality reduction, developing accurate and cost-effective method for screening and diagnosis of this cancer has occupied the minds of physicians for years. Previous studies reported high diagnostic accuracy for Pap smear, VIA, and VILI with respect to cervical cancer. Moreover, not all developing countries have access to colposcopy. Therefore, this study was attempted to compare these three tests with colposcopy in terms of diagnostic value.

Methods

This diagnostic study was conducted on 328 women referred to Shahid Sadoughi Clinic for colposcopy. At the first step, Pap smear was performed for those who did not undergo this test previously. Then, all the participants underwent VIA and VILI tests according to the known protocol. Next, colposcopy was conducted for all the participants, biopsy sample was obtained, and histological features were studied. Finally, the results were compared based on statistical indicators.

Results

Sensitivity of 91.9% and specificity of 53.6% were obtained when Pap + VILI + VIL test results were compared with biopsy.

Positive predictive value of 20% and negative predictive value of 98% achieved.

A diagnostic accuracy of 58% was gained. In case of positive CIN II results and higher degrees of CIN, the findings were as follows when colposcopy results were compared with biopsy ones:

Sensitivity of 86.5% and specificity of 95.5% were obtained.
Positive predictive value of 71.1% and negative predictive value of 98.2% were acquired.

A diagnostic accuracy of 94% was attained.

**Conclusion**

According to our findings, Pap+ VIA+ VILI test results are comparable with colposcopy ones in terms of diagnostic accuracy (73% and 75%, respectively). Therefore, Pap+ VIA+ VILI test is recommended as a competent alternative to colposcopy in developing countries.

**References**


8. Perkins RB, Langrish SM, Stern LJ, Figueiroa J, Simon CJ. Comparison of visual inspection and Papanicolau (PAP) smears for cervical cancer screening in


HPV analysis improves the PPV of Atypical Glandular Cells

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Background / Objectives

The finding of atypical glandular cells (AGC) is important for the possible prevention of endocervical adenocarcinoma (ADCA). This diagnosis group is quite unspecific and includes several cases with reactive conditions as well as dysplastic squamous lesions. The purpose of this study is to determine how reflex HPV analysis may improve the positive predictive value (PPV) of AGC.

Methods

During 2014 - 2015, altogether 385 LBC samples (ThinPrep®, Hologic) were diagnosed as AGC. Reflex HPV analysis were performed by the Cobas 4800 platform (Roche Diagnostics). Histological follow-up was available in 206 (54%) cases - 105 (51%) of these containing HR-HPV.

Results

The HPV positive group contains 3 cases of cervical ADCA and 15 cases of adenocarcinoma in situ (AIS) together with 51 cases of high grade squamous intraepithelial lesion (HSIL). The PPV for a lesion to treat was 69/105 (66%). The corresponding figures for the HPV negative group was 3 endometrial carcinomas, 1 metastatic breast carcinoma and 2 HSIL, giving a corresponding PPV of 6/101 (6%). HR-HPV was found in 69/71 cases with cervical lesions to treat (sensitivity 97%).

Conclusion

The results highlight the importance of combined cytology and HPV analysis. HPV defines AGC cases with an exceptionally high PPV for high grade lesion to motivate follow-up.

References


EVALUATION OF A HOST DNA METHYLATION PANEL IN A HIGH HPV PREVALENCE COHORT

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Background / Objectives

Today there is a strong agreement in the scientific community regarding the hr-HPV testing superiority in cervical cancer primary screening versus cytology. However, due to the limited specificity of hr-HPV testing, new biomarkers are needed in order to triage the positive cases, avoiding overtreatment and excessive referral to colposcopy. DNA methylation (viral and host) has been proposed as a promising strategy. This work aims to evaluate clinical specificity and sensitivity of a host methylation panel proposed by Hansel et al., in 2014 with some modifications (GynTect®).

Methods

A maximum of 100 consecutive PreservCyt® samples diagnosed with LSIL and histology follow-up were selected from the routine diagnostics at LAP Unilabs Porto. In order to access sensitivity some other samples with histologically confirmed CIN3+ lesions were also included. All results were compared with previous cytology, HPV status, CINtec® Plus results and histology data available for that sample. Information regarding the evaluated sample, patient information and data regarding other clinical history related to that patient were taken from the laboratory database to allow the clinical significance of the results to be assessed. All data stored for the evaluation was anonymized. The gold standard is histologically confirmed cervical intraepithelial neoplasia (CIN) grade 3+. Sensitivity and specificity of each of the triage tests was calculated based on disease defined as CIN 3+ (p16 stain confirmed).

All DNA methylation panel testing were performed in the Roche cobas®480z analyzer (component of the cobas®4800 system).

Results

Data is being collected, but the results are not yet fully available.

Conclusion

Preliminary data confirms the capability of the markers to detect high grade disease, with a low false-positive rate.

References

https://doi.org/10.1371/journal.pone.0091905)
Biological risk factors associated with methylation positive and negative high-grade cervical lesions – clinical study

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Background / Objectives

Cervical carcinogenesis is a multistep process which starts with an acquisition of high-risk HPV infection. Multiple biological and behavioural risk factors affect the progression towards severe lesions. The most pronounced risk factors include viral factors, immunosuppression, co-occurrence of other sexually transmitted infections (STI), genetic polymorphism of tumour suppressor genes (TSG), and smoking. Risk factors contribute to the transition from incident to permanent HPV infection and from persistent infection to high-grade lesion.

HPV-induced methylation silencing of TSGs is believed to be a sign of high-grade lesions carrying higher risk of short-term progression into invasive stadium. While almost all cases of invasive carcinomas have methylated promotors of specific TSGs, less advanced severe cervical lesions (HSIL) are methylated only in 60-90 % cases. Morphology or biomarkers reflecting differences between methylated and unmethylated HSIL lesions are scarcely described in the literature.

In our study, we focused on exploring biological risk factors in methylated and unmethylated HSIL lesions, namely co-occurrence of other STIs and presence of certain HR-HPV genotypes.

Methods

108 residual samples of liquid-based cytology of Czech women with histologically confirmed high-grade cervical lesion were analysed with

1) Precursor M kit (Self-screen) to assess methylation status of tumour-suppressor genes CADM1, MAL, and has-miR-124, related to cervical carcinogenesis

2) LINEAR ARRAY HPV Genotyping Test (Roche) and type-specific PCR targeting E6 and E7 viral oncogenes to reveal specific HR-HPV genotypes

3) Allplex STI Essential Assay (SeeGene) to discover possible co-occurrence of 7 most common microbial pathogens responsible for cervicitis

Methylated and unmethylated HSILs were evaluated in two categories, first presence of HPV genotypes 16,18 and 45, and second co-occurrence of any microbial pathogen.

Results
36% of HSIL lesions in our study had negative methylation status. At least one microbial pathogen was detected in 50% of HSIL lesions but there was no significant difference between methylated and unmethylated groups. HPV types 16, 18, and 45 were detected more often among methylation positive samples but this finding was not statistically significant.

Conclusion

There is no difference in STIs co-occurrence between methylation negative and positive HSILs. HPV types 16, 18, and 45 occur more often in methylation positive HSILs but also infection of other HR-HPV types might result in methylation of TSG’s promoters. Further studies are needed to confirm that the methylation status differentiates biological early lesions from advanced HSILs.

References

1) CADM1, MAL and miR124-2 methylation analysis in cervical scrapes to detect cervical and endometrial cancer. Journal of Clinical Pathology
THE INTERACTION BETWEEN HPV INFECTION AND BACTERIAL MICROBIOTA IN PLACENTA, CERVIX AND ORAL MUCOSA

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Background / Objectives

Objective. We aimed to investigate whether an existing HPV infection has influence on the bacterial microbiota composition in the placenta and cervix as well as in the maternal and infant oral mucosa.

Methods

Materials and methods. This study is a nested case-control study based on samples collected in the prospective Finnish Family HPV Study. Total 39 families were selected for this study based on placenta HPV status, the mode of birth and availability of samples (13 cases with HPV positive placenta and 26 controls with HPV negative placenta of which 13 were obtained through vaginal delivery and 13 by Caesarean section). The corresponding maternal cervical and oral and infant oral samples were selected for analyses.

HPV DNA genotyping of 24 different genotypes (6 low-risk and 18 high-risk types) of the samples was conducted using Multimetrix ® assay (Multimetrix, Regensburg, Germany). Microbiota composition and diversity was characterized by 16S rRNA gene sequencing (V1-V3 region, illumina protocol, Illumina, San Diego, CA, USA).

Results

Results. HPV DNA was found in 23% (9/39) maternal cervix, 33% (13/39) maternal oral, and 45% (18/40, included one set of twins) infant oral samples. HPV16 was the most frequent type found in all groups studied (54% of placenta, 22% of cervix, 54% of maternal oral and 39% of infant oral samples).

In maternal mouth, HPV positive samples displayed significantly higher richness (Chao1 index) of bacterial microbiota (p=0.032) but no difference in Shannon index. HPV status did not influence microbial diversity and richness in the other samples.
The HPV positive cervix harboured significantly more *Adlecreutzia* (p=0.048), *Mycoplasma* (p=0.048) and *Gemella* (p=0.0058) genus as compared to HPV negative cervical samples. In maternal oral samples, *Selenomonas* spp. was significantly increased (p=0.012) in HPV positive individuals whereas the amount of *Propionibacterium* (p=0.026) and *Staphylococcus* (p=0.049) were increased in HPV positive infant oral samples. In the placenta, *Lactobacillus* (p=0.076) were slightly increased in HPV positive samples compared to placenta HPV negative.

**Conclusion**

**Conclusion.** HPV infection is associated with altered bacterial microbiota composition in the placenta and mouth. Whether the changes in bacterial microbiota predispose or result from HPV remains to be determined in future studies.
VAGINAL MICROBIOTA AND PAP SMEAR

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Background / Objectives

The bacteria in the human vagina have an important role in maintaining general health and protecting host from pathogenic microbes. Our knowledge about vaginal microbiota and its complexity has expanded vastly after development of novel culture-independent methods. Yet the big picture of vaginal microbiota remains the same as when Döderlein first found Lactobacillus from vagina.

In recent studies, the human papilloma virus (HPV) infection and its clearance rate been linked with vaginal microbiota type and bacterial vaginosis (BV) [1,2]. This emphasizes the need for better understanding of the function of different microbiota types and their interplay with the host.

Methods

We sampled 50 healthy Finnish women during routine Pap smear screening for cervical cancer in Helsinki, Finland. We collected an extensive background questionnaire and swabs for microbiota and HPV analysis. The Pap smears were reanalyzed to classify microbiota features visible to microscope. For bacterial community profiling, we used Illumina HiSeq platform to sequence hypervariable V3-4 regions of the 16S rRNA gene. For estimation of different strains among observed species we used minimum entropy decomposition (MED) [3] and oligotyping [4] and for functional analysis we used PICRUST [5] and other similar methods.

Conclusion

We have just started to analyze the data. The preliminary analysis identified interesting associations between the microbiota, socioeconomic factors and on the other hand between the Pap smear microscopy and sequencing. The results and conclusions will be presented at the conference.

References


**P18-01**

**DEVELOPMENT AND VALIDATION OF AN OPTIMIZED HPV COMPETITIVE LUMINEX IMMUNOASSAY (9-PLEX) AND HPV IgG ANTIBODY DETECTION LUMINEX IMMUNOASSAY (9-PLEX) SUPPORTING CLINICAL SEROLOGY TESTING FOR GARDASIL-9**

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**Background / Objectives**

Two multiplex Luminex immunoassays are used to assess antibody responses in MSD Gardasil-9 clinical trials: (1) The primary immunoassay is the HPV 6, 11, 16, 18, 31, 33, 45, 52, 58 competitive Luminex immunoassay (HPV-9 cLIA) and (2) the secondary assay, the HPV 6, 11, 16, 18, 31, 33, 45, 52, 58 total IgG Luminex immunoassay (HPV-9 IgG assay), is used for supportive analyses. Recently, both assays were re-developed, and the optimized assays were validated and approved by the Center for Biologics Evaluation and Research, U.S. Food and Drug Administration. In addition, the assays were formally bridged to the previous assay versions to assess serostatus cutoffs (SSCO) and the impact of the changes on persistence studies.

**Methods**

The optimization of the assays included assessment of the following parameters: VLP coating concentration, wash buffer, Luminex microspheres, serum sample and reference standard diluent, reference standard starting dilution and titration series, and vendor and concentration of the PE-labeled antibodies. For both assays, the validation studies evaluated various performance parameters including intra-assay precision (repeatability), intermediate precision, linearity, relative accuracy/dilutability, and limits of quantitation. For the bridging study, individual patient sera from an MSD clinical trial, including day 1, month 7, and month 36 serum samples from 100 subjects, and an additional 50 day 1 samples, were used to compare measured concentration results to the historical values.

**Results**

Analysis of the validation data indicates that the optimized HPV-9 cLIA and IgG assays are accurate, specific, and precise throughout the quantifiable range for each HPV type. Results of the bridging study indicate that there is a strong positive linear
association between the assay versions. For both HPV-9 cLIA and IgG assays, the SSCOs were adjusted to align seropositivity rates between assay versions.

Conclusion

Optimization of the assay, including the elimination of antibody-depleted human serum in the assay buffer and increasing the starting dilution from 1:4 to 1:10, led to an improvement in the dilutability of the HPV-9 cLIA (within 1.25-fold per 10-fold dilution) relative to the prior version. For both HPV-9 cLIA and IgG assays, the strong positive linear association between the previous version and optimized version allow for immunogenicity assessments of long-term follow-up studies across assay versions.
A NEW GENERATION OF VALIDITY TESTING FOR ONCOPROTEIN-BASED CERVICAL CANCER SCREENING

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Background / Objectives

HPV is known to infect basal keratinocytes found within the cervical transformation zone. Promising new HPV tests are based on the detection of viral oncoproteins of hrHPV types, but their diagnostic capabilities may be limited without a way to assess specimen validity. Hence, there is a need to reduce false negative results of these tests due to unreliable sampling. Here we describe a new assay that captures cytokeratins 5, 8 and 18 from potential target cells as a means of normalizing cervical specimens.

Methods

A keratin 5/8/18 sandwich ELISA – recomWell Keratin 5/8/18 - was developed for detection of cells located within or originating from the cervical transformation zone. Content of different cell types was validated microscopically. Suitable for measurement of Keratin are liquid-based cytological samples in PreserveCyte.

Results

The Keratin ELISA was successfully validated with cell lysates of HPV positive and negative cell lines of cervical origin. The proof of concept was shown by measurement of well characterized clinical samples. In 335 HPV positive samples of all stages of CIN, Keratin 5/8/18 could be detected with a similar signal distribution when compared to 1484 normal samples (OD 0.96 +/-0.17). 94.5% of all samples and 96.1% of normal samples showed signals for Keratin 5/8/18 above cut off. On the contrary, 90.4% of samples with CIN2+ and 89.1% with CIN3+ were positive for Keratin 5/8/18.

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 validated the expression of these Keratins in individuals with HPV-induced dysplasia.
and found differences in the proportion of valid samples between healthy woman and those which developed CIN2+. Furthermore, recommendations for interpretation of the results of the combined test systems (validity testing by recomWell Keratin ELISA and HPV E7 oncoprotein testing by recomWell HPV 16/18/45) were set.

The recomWell Keratin 5/8/18 allows validity testing of cervical samples by detection of potential HPV target cells and could therefore be a means to decrease the rate of false negative HPV results due to unreliable sampling.
ESTABLISHMENT OF THREE-DIMENSIONAL ORGANOTYPIC RAFT CULTURES CLOSELY MIMICKING HPV-TRANSFORMED CERVICAL LESIONS IN AN EPITHELIAL CONTEXT

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Background / Objectives

HPV-transformed cancer cell lines have so far been intensively studied and characterized in monolayer cultures. To develop novel medical treatment options a three-dimensional (3D) model of HPV-transformed cells in their natural context is of superior importance when analyzing drug effects on these cells. We intended to create a 3D organotypic epithelial raft culture resembling HPV-induced (pre-)cancerous lesions. This will allow better evaluation and understanding of the effects of novel therapeutic options on HPV-transformed cancer cells in their natural context as well as on surrounding healthy keratinocytes.

Methods

A dermal equivalent comprising a fibrin gel containing human fibroblasts embedded in a tissue scaffold was created in a deep-well plate. A combination of primary human keratinocytes and cervical HPV-transformed cancer cell lines was then seeded on the dermal equivalent and grown to full confluency over the course of 24 hours. Epithelial differentiation of the keratinocytes occurred over the course of 2 weeks. The cultures were subsequently harvested. Histological slices were prepared and stained with hematoxylin & eosin. Furthermore, combined p16INK4a/Ki67 immunohistochemical staining as well as combined Keratin 14/Keratin 7 immunofluorescence were performed.

Results

After two weeks of culture we observed a fully differentiated epithelium comprising healthy keratinocytes and clearly distinguishable lesions consisting of HPV-transformed cancer cells. p16INK4a/Ki67 immunohistochemical staining allowed for a clear distinction between normal keratinocytes and HPV-transformed cells with only HPV-transformed cells staining positive for both markers. Likewise, Keratin 14/Keratin 7 immunofluorescence granted a specific identification of the HPV-transformed cervical cancer cells. These transformed lesions, established with various HPV-transformed cell lines, resemble actual lesions found in a natural environment.

Conclusion
A 3D organotypic raft culture growing HPV-transformed cells in a more natural setting was established, providing an ideal model to study effects of future therapeutic approaches.
Retrospective study of sensitivity, specificity, positive predictive value and negative predictive value of cytology in liquid medium, HPV DNA test and genotyping for HPV16, in different cervical cancer screening scenes

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Background / Objectives

Estimation of sensitivity (SE), specificity (SP), positive and negative predictive value (PPV/NPV) of cytology in liquid medium, HPV DNA test and partial genotyping for HPV16 in women older and younger than 30 years old in 3 screening periods: 2010-13, 2014-5 and 2016 (Huesca General Hospital, Spain).

Methods

Our target population is 54.372 women. Cytologies are performed in liquid medium. HPV determination is performed by COBAS 4800 Roche platform. From 2010 to 2013, screening was based on triennial cytology and HPV DNA test in ASC-US; during 2014-2015 period on co-testing in > 30 years-old women, and from 2016 on HPV DNA test with reflex cytology and partial genotyping for HPV16-18. The burden of disease is based on colposcopy biopsies.

Results

<table>
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<th>Table 1. Cytology in liquid medium.</th>
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<tr>
<td>2010-13 (n=197)</td>
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<td>SE&lt;30 years-old</td>
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<td>SE&gt;30</td>
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<td>PPV&lt;30</td>
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<td>PPV&gt;30</td>
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NPV $<$ 30 | 95.5% | 73.7% | 50%
NPV $>$ 30 | 73.2% | 69.4% | 63.5%

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<th>Table 2. HPV DNA test</th>
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<td>SE $&lt;$ 30 years-old</td>
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<td>SE $&gt;$ 30</td>
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<th>Table 3. HPV DNA type 16</th>
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<td>SE $&lt;$ 30 years-old</td>
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<td>NPV $&lt;$ 30</td>
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<td>NPV $&gt;$ 30</td>
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**Conclusion**

Cytology shows a progressive reduction of sensitivity and specificity in $<$ 30 years-old women. There are also marked variations in specificity and NPV in women $>$ 30. These changes may be due to new screening paradigms and to the incorporation of younger pathologists. On the other hand, PPV increases with the incorporation of HPV to screening.
Sensitivity for HPV DNA test is around 100%, regardless of age and analyzed period. Its specificity has increased with the systematic HPV DNA test (following a standard protocol) and with patient selection following consensus guidelines.

Positivity for HPV16 is associated with a decrease in sensitivity, specially in women > 30 years-old, coupled with a spectacular increase in specificity, mostly in women > 30. Likewise, a moderate increase in PPV is corroborated, with no relevant age differences. In addition, there is a slight to moderate decrease in NPV, regardless of age and screening periods.

The explanation to low specificity of HPV DNA test may be that 2/3 are positive to some of the types NO16-18.

The high sensitivity and low specificity of the HPV DNA test make us incorporate more specific techniques that reduce unnecessary colposcopies. Perhaps dual staining help us to refine derivation scheme.

References

Conization using electrosurgical conization with cold coagulation for cervical intraepithelial neoplasia: a feasible treatment with a low risk of residual disease

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Background / Objectives

Objective: This study was performed to evaluate the significance of positive resection margins (RMs) of electrosurgical conization with cold coagulation as definitive treatments for patients with cervical intraepithelial neoplasia (CIN).

Methods

Methods: We retrospectively reviewed 306 patients who underwent electrosurgical conization with cold coagulation for CIN treatment at our institute from August 2005 to December 2016. A right-angled triangular loop in a single pass followed by a cold coagulator (120°C) to the cone bed for 10 to 20 seconds was used. Patients with positive RMs were underwent pap smear, human papilloma virus (HPV) DNA testing, and endocervical curettage after 3-6 months without additional treatments. Patients with margin positive invasive carcinoma or adenocarcinoma in situ (AIS) recommended hysterectomy, firstly. Pathologic reports and clinical data were obtained and evaluated.

Results

Results: Histopathological evaluation of electrosurgical conization materials revealed the presence of CIN I in 54, CIN II/III in 241, AIS in 3, and invasive carcinoma in 8 (microinvasive/adenocarcinoma, 7/1, respectively) patients. Margins were positive in 41 (13.4%) cases; 0 in CIN I, 37 in CIN II/III (15.4%), 1 in AIS (33.3%), and 3 in invasive carcinoma (37.5%), respectively. Twenty-eight patients had positive endocervical RMs, while thirteen patients had positive exocervical RMs. In this series, there were no cases with simultaneous positive endocervical and exocervical RMs. Six patients with positive margins were lost to follow-up. Two CIN cases with positive RMs revealed 1 CIN I and 1 CIN III at first follow-up. Three microinvasive carcinoma cases revealed 1 no residual disease, 1 CIN I, and 1 CIN II after hysterectomy. However, one adenocarcinoma case without positive RM and one AIS case with positive RM revealed no residual disease after hysterectomy. Totally, four out of 300 patients (1.3%) who underwent electrosurgical conization with cold coagulation had residual diseases.

Conclusion

Conclusions: These results suggest that electrosurgical conization with cold coagulation is
a feasible treatment for CIN cases with a low risk of residual disease. Patients who are diagnosed with CIN preoperatively could be followed up without additional treatments in spite of positive RMs.

References

Key Words: Cervical intraepithelial neoplasia, Microinvasive carcinoma, Electrosurgical conization, Cold coagulation, Resection margin
P20-03
Evaluation and correlation of primary histopathological diagnosis of targeted biopsy till the final histopathological diagnosis following diagnostic and therapeutic process of leep conization

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Background / Objectives

Evaluation of consistency of the final histopathological diagnosis following diagnostic and therapeutic process of leep conization till the primary histopathological diagnosis following targeted cervical biopsy.

Methods

The analysis included 540 patients, in whom leep conization was performed due to incorrect result following targeted biopsy – SIL or discrepancy of cytological – histopathological results. Targeted biopsy and leep conization were performed by doctors with years of experience. Colposcopy was performed using stereoscopic colposcope (Olympus OCS-500). Colposcopic pictures were evaluated according to Reid’s index, regarding the margin and acetowhitening, iodine negative test and vascularization.

Results

The most frequent histopathological diagnosis following targeted biopsy and leep conization were HGSIL-CIN 2 type changes, which comprised 36% and 34% respectively and HGSIL-CIN 3 type changes which comprised 21% and 27% respectively. Consistency of histopathological results of targeted biopsy compared to the final result following leep conization comprised 94% for HGSIL type changes.

Conclusion

Targeted cervical biopsy and leep conization show very high consistency in terms of histopathological results.
P20-04
WHAT’S BEHIND LSILs?


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Background / Objectives

Low grade intraepithelial lesion (LSIL) is the second most common anomaly on cervical cytology. Despite the low risk of progression to carcinoma, 10-25% have a histological diagnosis of cervical intraepithelial neoplasia grade 2 or higher (CIN2+). Therefore, the challenge these lesions present is to identify the patients at risk of developing premalignant or malignant lesions. Our aim was to evaluate demographic characteristics, colposcopic findings and clinical follow-up of patients with LSIL on cervix cytology.

Methods

Retrospective longitudinal study of the patients referred to our Cervical Pathology consultation during January-December 2014 (n=356). Colposcopic classification was performed according to 2011 IFCPC nomenclature and histologic classification was divided in three groups: No displasia, CIN 1 and CIN2+. Statistical analysis was performed using SPSS ® v.21.

Results

LSIL was the indication for referral of 36% (n=128) of patients. The mean age at referral was 38.46±10.49 years, 14.1% (n=18) were post-menopausal, 64.6% (n=52) had >1 sexual partners, 20.3% (n=16) were smokers, 63.6% (n=70) were contraceptive pill users and 39.6% (n=40) were positive for high risk HPV. 96.4% (n=124) had a colposcopy done upon admission: 33.9% (n=42) were normal, 62.1% (n=77) had grade 1 findings and 3.9% (n=5) had grade 2 findings. Within those with grade 1 findings, biopsy revealed no displasia in 63.6% (n=49), CIN 1 in 27.2% (n=21) and CIN2+ in 9.1% (n=7). Within the group of grade 2 findings, one case presented no displasia, one case presented a CIN1 and 3 cases presented CIN2+. There was a significant association between grade 2 colposcopic findings and high grade histologic lesions (OR 13.714; IC 1.978-98.065). Regarding the therapeutic approach, CIN1 lesions underwent expectant management in 63.6% (n=14) and destructive therapy in 36.4% (n=8). All CIN2+ lesions were submitted to excisional therapy. During a 6-24 month follow-up period, there were no de novo high grade lesions. There was no case of cervical cancer in our sample.

Conclusion

In accordance with the literature, LSILs were more prevalent in premenopausal women, with a higher number of sexual partners and a high prevalence of high risk HPV. Despite traducing mostly low grade histologic lesions, CIN2+ was present in
12.3%. The presence of grade 2 colposcopic anomalies correlated with high grade histologic lesions, reinforcing the importance of colposcopy in the surveillance of these patients.
P20-05
CYTOLOGICAL CHANGES IN WOMEN UNDER 25 YEARS


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Background / Objectives

In Portugal, cervix cancer has an overall incidence of 13.5 in 100000 women, having its peak incidence in the 4th decade of life. The mortality rate decreased due to an organized screening program, comprising women between 25 and 65 years of age. Organized screening in women under 25 years has not demonstrated any reduction of the incidence or mortality rates. Nonetheless, these women are still screened, occasionally. Based on this fact as well as on the increased occurrence of false-positive screenings in women under 21, it is perfectly adequate that cytological changes or HPV tests are not highly valued. Similarly, between the ages 21 to 25, the follow up of these changes should globally be less invasive, adopting a wait-and-see attitude.

The purpose of this study is to compare the risk factors for cervical cancer between women under 25 and the remaining sample. In this age group, we intend to evaluate colposcopy findings, treatment and follow up.

Methods

Retrospective study of women referred to the Cervical Pathology practice in our Department in 2014, through review of their clinical files (n=356). Statistical analysis made through STATA® v.13.1.

Results

Of the total sample, 5.6% (n=20) of women were younger than 25 years. These presented a statistically significant greater number of partners and an earlier start of their sexual activity when compared to the remaining sample. The most common cytological alteration was low squamous intraepithelial lesion (LSIL) (65%). Upon the first appointment, 30% had a cytology done and 90% a colposcopy, 22.2% of which were normal. Of the 77.8% that had cytological alterations, 78.6% were subjected to a biopsy. Regarding the patients referenced for an LSIL injury, of whatever extent, 100% of the colposcopies found minor anomalies, whereas of those that underwent a biopsy 100% of the histology revealed minor injuries. The most serious injury detected was cervical intraepithelial neoplasia grade 2 (5.5%).

Conclusion
The alterations resulting from the occasional screening in women under 25-years of age continue to account for an increased number of medical appointments. Moreover, these patients present increased risk factors when compared to older patients with cervical pathology. Colposcopy proved to be the favoured exam for the initial approach, with a high number of biopsies. A high concordance between cytology, colposcopy and histological findings was verified, which strongly supports that a less invasive attitude may be adopted. Scheduling appointments with a greater time interval might also be a strategy to avoid over-treatment.

References

Colposcopy Evaluation at the Time of LEEP May Avoid Unnecessary Treatment

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Background / Objectives

The Loop Electrosurgical Excision Procedure (LEEP) is the mainstay technique for the treatment of high-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia (SIL/CIN). The use of colposcopy during LEEP improves the accuracy of treatment and reduces the risks of the procedure. However, its possible benefits in relation to the identification of patients with no lesion at the time of LEEP have not been established. The aim of the study was to assess the accuracy of colposcopy evaluation at the time of the LEEP to identify women with a previous confirmatory diagnosis of SIL/CIN with the absence of dysplasia in the cone specimen.

Methods

We prospectively recruited 162 women undergoing LEEP for histological HSIL/CIN2-3 or LSIL/CIN1 with HSIL cytology showing a fully visible squamocolumnar junction in the colposcopy evaluation at the time of LEEP. At the referral visit cervical cytology, human papillomavirus (HPV) detection and genotype identification, digital colposcopy, size of the colposcopical lesion, and one or more biopsies of the transformation zone were obtained. The uterine cervix was colposcopically evaluated intraoperatively.

Results

Thirty-four women (21.0%) had a normal colposcopy evaluation at the time of the LEEP (study group), while the remaining 128 women showed abnormal findings (control group). Absence of SIL/CIN in the LEEP specimen was confirmed in 28 of the 34 (82.3%) women in the study group and 8 of the 128 (3.1%) women of the control group (p<0.001). A normal colposcopy evaluation at the time of LEEP, lesion size ≤12mm² at the referral colposcopy and HPV genotypes other than 16 or 18 were associated with the absence of CIN in the univariate logistic regression, but only a normal colposcopy evaluation remained significant in the multivariate analysis. A normal colposcopic evaluation at the time of LEEP increased the risk of absence of lesion in the cone specimen 229-fold compared with cases presenting an abnormal colposcopy (95%CI: 33.8-1555.1; p<0.001). The colposcopy evaluation at the time of LEEP had a sensitivity of 87.5% (95%CI: 71.9-95.0) and a specificity of 95.4% (95%CI: 90.3-97.9) to predict the absence of SIL/CIN in the LEEP specimen.
Conclusion

These data show that colposcopy evaluation at the time of LEEP can accurately identify the absence of SIL/CIN before treatment. Thus, the performance of excisional procedures for the treatment of SIL/CIN under direct colposcopy vision should be recommended. Moreover, small lesions and HPV types other than 16 and 18 may point to patients with a higher probability of having a normal colposcopy evaluation at the time of treatment, indicating which women can forgo the treatment.

References


BACKGROUND / OBJECTIVES

Quality Assurance (QA) is a way of maintaining a high quality of health care services by constantly measuring the outcome of clinical practice. QA is becoming increasingly important in health care. Nevertheless, there are no specific quality requirements for colposcopy and colposcopy-guided treatments in Switzerland and many other European countries. The European Federation of Colposcopy (EFC) conducted a five-round Delphi consultation to define six quality indicators for colposcopic practice. These indicators were slightly adapted at the EFC general meeting in Paris in January 2017.

METHODS

We retrospectively evaluated these quality indicators in our colposcopy clinic during the period from January 2015 to December 2016. The six indicators and corresponding targets are (1) documentation of the transformation zone type (100%); (2) percentage of cases having a colposcopic examination prior to treatment for abnormal cervical cytology (100%); (3) percentage of conisations (diagnostic or therapeutic biopsies) with cervical intraepithelial neoplasia (CIN) 2+ (≥85%); (4) percentage of excised lesions with clear margins (≥80%); (5) number of colposcopies personally performed each year with low grade/minor changes (≥50); and (6) high-grade/major lesions (≥50).

RESULTS

From January 2015 to December 2016, 148 conisations were performed at our colposcopy clinic. The transformation zone type was documented in nearly every colposcopy (99.3%, 147/148). 99.3% (147/148) had a colposcopic examination prior to treatment for abnormal cervical cytology and 87.3% (130/148) of conisations showed CIN 2+ in diagnostic or therapeutic biopsies. 43.2% (64/148) of excised lesions had clear conisation margins. Each colposcopist at our clinic performed more than 50 colposcopies with low grade/minor changes and high-grade/major lesions per year.

CONCLUSION
Adopting the quality indicators recommended by the EFC offers the possibility to evaluate the performance of colposcopists and provide a benchmark system to secure performance both nationally and internationally. By applying these quality indicators to our retrospective data, we identified our strengths and weaknesses, which will enable us to make future improvements in the care of our patients.

References

A comparison of loop electrosurgical excision procedure using a ring-shaped loop versus a right-angled triangular loop

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Background / Objectives

Objective To compare the resection margin (RM) status and postoperative severe hemorrhage (SH) using different loop electrosurgical excision procedure (LEEP) techniques for cervical intraepithelial neoplasia (CIN) 2/3 treatment.

Methods

Study Design We retrospectively reviewed 278 patients who underwent LEEPs for CIN 2/3 treatment at our institute between 2005–2014. In type A surgery (N=148), a ring-shaped loop was used. If the first pass failed to remove the entire lesion, separate loop excisions for the intracervical portion were performed. In type B surgery (N=130), a right-angled triangular loop in a single pass was used. Surgical outcomes and postoperative SH were compared between the two groups. Logistic regression analysis was performed to identify the independent predictors of RM status.

Results

Results The mean LEEP depth was larger after type A surgery (2.2 vs 2.0 cm, respectively; P=0.04). Type B surgery showed lower rate of 30-day postoperative hemorrhage (13.8% vs 26.4%, P <0.05) and higher rate of negative RM (68.9% vs 82.3%, P<0.05). Multivariate analysis identified the surgery type [P=0.01, OR=0.45 (0.24-0.83)] and a postoperative pathological diagnoses of CIN3 [P=0.01, OR=2.53 (1.22-5.26)] as independent risk factors for positive RM.

Conclusion

Conclusions LEEPs using a right-angled triangular loop could reduce positive RMs.

References

Keywords cervical intraepithelial neoplasia, LEEP, resection margin, postoperative hemorrhage
Post-Coital Bleeding (PCB) as a Predictor for Cervical Pathology: A cross Sectional Study

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Background / Objectives

Post-coital bleeding (PCB) is a disturbing gynecological symptom that may be a concern for both patient and physician, and its reported prevalence varies from 0.7%–9% among menstruating women. PCB may reflect different benign conditions such as infectious morbidities, but can also indicate the presence of cervical cancer. Colposcopy has been suggested as the appropriate investigation tool required for ruling out cervical cancer, or other pre-malignant pathologies; however the literature is not decisive in the management recommendations. The objective of this study was to evaluate the role of PCB in predicting cervical pathology.

Methods

A cross-sectional study using the computerized database of HMO encompassing 2 million insured patients. The research was approved by the local REB committee (number 25/2106). All of PCB cases were identified through a computerized query relating to the years 2012-2016. Further, patients’ records in a single center were investigated and the following variables were assessed: age, marital status, ethnical background, gravity, parity, BMI, smoking, socio-economic status, past history of cervical pathology, PAP smear result and colposcopy evaluation. Colposcopy reports were reviewed for findings, required biopsy and biopsy results. For the purpose of this study we included non-pregnant patients between 18-50 years.

Results

Incidence/100,000 patients during the study period ranged 326.1-565.9 cases/year. Among investigated records, mean age was 32±7.9 years, mean BMI: 24.0±4.3, 53% were married, 49.1% gave birth, 17% were smoking and 18.7% presented with a background medical diagnosis. 8% of PCB cases had an abnormal PAP smear in the preceding year. All sample cases went through a colposcopy by a single practitioner; 201 (48.9%) requiring a biopsy. Biopsy results were as following: 44 (21.9%) normal tissue, 25 (12.4%) cervical polyp, 68 (33.8%) cervicitis, 61 (30.3%) HPV-related/CIN 1/condylomas, 2 (1.0%) CIN-2/3 and 1 case (0.5%) of
carcinoma. The positive predictive value for HPV-related pathology was 15%, and for high-grade lesions (CIN-2/3 and carcinoma): 0.7%. In a multivariate logistic regression analysis, parity and the presence of a pathological PAP smear (P value 0.02, OR 0.39 and P value 0.01, OR 3.3 respectively) were significantly related to HPV-related cervical pathology.

**Conclusion**

PCB is a common gynecological complain with relatively high prevalence of HPV-related pathologies. Although high grade lesions are rare, we recommend considering colposcopic evaluation in those women.

**References**
Progression of cervical intraepithelial neoplasia in pregnancy

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Background / Objectives

The aim of the study was to analyze the regression, persistence, and progression of cervical intraepithelial neoplasia first diagnosed during pregnancy, in order to assess the suitable management of such lesions for the pre- and postpartal period.

Methods

In the course of this study the cases of 138 pregnant women who presented with pathological cervical findings at the Dysplasia Clinic of the University Medical Center Hamburg Eppendorf between the years of 2011 and 2017 were retrospectively analyzed. Differential colposcopy, a cytology, a biopsy and as appropriate a HPV test were performed on all patients. In the case of CIN diagnosis regular follow-ups were carried out. The initial histopathological findings were compared to those of the postpartal period.

Results

A total of 138 pregnant women of the median age of 31 years (range 19-41) with colposcopic evidence of cervical dysplasia (n=15) or suspicious cytology (n=53 with PAP IIID, n= 70 with PAP IVa/b) were included. On average the patients first presented in the 17th (range 5-31) week of pregnancy and were followed-up every 8 weeks. No progression to carcinoma was diagnosed in any of our patients and no woman had to be subjected to a conisation during the course of pregnancy. 60 patients with initial CIN diagnosis during pregnancy were presented for a scheduled postpartal exam, where 16.7% (n=10) showed a partial regression of CIN, while 40% (n=24) showed a complete regression of CIN. 33.3% (n=20) were diagnosed with persistent findings of CIN. In 10% (n=6) of cases progression to severer CIN not however to a carcinoma had occurred. All in total 34 patients were operated on postpartally by conisation and endocervical curretage.

Conclusion

CIN lesions during pregnancy have a prepartal slight progression and postpartal a higher tendency for regression. After the exclusion of an invasive procedure, the definitive treatment can be postponed with little risk to the postpartal period. The necessity of a check-up every 8 weeks after the detection of a high-grade lesion (CIN 2-3) during pregnancy can not be deduced/inferred from these findings.
ASSOCIATION BETWEEN VAGINAL MICROBIOME, HIGH RISK HPV PROFILE, HPV E6/E7 RNA EXPRESSION AND SEVERITY OF CERVICAL PRECANCEROUS LESIONS

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Background / Objectives

Role of persistant HPV infection in development of precancerous lesions is essential and multiple HPV infection correlation with E6/E7 RNA expression is shown in some studies (Anderson 2012). There are no studies in Latvia on HR HPV profile and E6/E7 RNA expression in patients with cervical intraepithelial neoplasia.

Methods

49 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 July 2016-February 2017 were included in the study. Results of vaginal pH, native microscopy of frontal vaginal fornix, material from cervix for presence of high risk (HR) HPV DNA types 16/18, 31, 33, 45, 58, HR-HPV E6/E7 common RNA and histology after punch biopsy taken under colposcopy control were analyzed for each patient. Vaginal pH was measured using Machery Nagel pH strips. Microscopic examination of wet mounts was interpreted according to Donder’s modification of Schröder’s classification. HPV types and HPV RNA were identified by real time PCR test.

Results

14 patients with low grade squamous intraepithelial lesions (LSIL), 32 with high grade SIL (HSIL), 2 patient with atypical squamous cells of undetermined significance (ASCUS) and 1 patient with atypical glandular cells of undetermined significance (AGUS) were included in the study. HR-HPV DNA was detected in 26 cases (HPV positive group), 23/26 was multiple HPV infection. The most common HPV type was HPV 58, which was isolated from 23 women, HPV16/18 was found in 16 patients, HPV 31 in 13 cases and 33 in 11 patients, HPV 45 in 11 patients. Elevated pH >4.4 was detected in 12/26 patients from HPV positive group and in 5/23 cases from HPV negative group (p=0.02). Lactobacillary grade III prevalence in both groups did not differentiate significantly in our study, 7 cases in HPV positive group and 8 cases in HPV negative group. HPV E6/E7 RNA expression was found in
26/26 cases of HR-HPV DNA positive group and in 13 cases of HPV negative group (p<0.01). Multiple HPV types were found in 19/34 patients with CIN 2+ histology. CIN2+ in histology reports was more likely correlate with HPV E6/E7 RNA expression in both groups: 21/26 patients in HPV positive group and 13/13 cases from HPV DNA negative group (p=0.05).

Conclusion

Our findings suggest a possible association between multiple HPV DNA, E6/E7 RNA expression and high grade cervical precancerous lesions, but we analyzed only 6 HR-HPV types and E6/E7 RNA prevalence in HPV negative group may be associated with other HR–HPV types. More detailed study will be required in future.
THE DISCOVERY OF A ANTITUMOR ACTIVITY OF THE ALKALOID ERYTHRALIN: INDUCTION OF APOPTOSIS IN SIHA CELLS BY ARRESTING THE CELL CYCLE AT THE G2-M PHASE

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Background / Objectives

Cervical cancer is the fourth leading cause of cancer death in women worldwide and persistent infection with a high risk human papillomavirus (HR-HPV) is the main etiological factor. Several studies have sought to identify compounds with selective activity for tumor cells that have an apoptotic mechanism of action, therefore it is important investigate bioactive new chemical entities mainly from biodiversity. Erythrina velutina (EV) is a plant native from Brazil popularly known as mulungu. Seeds and barks are used in folk medicine as sedative, anticonvulsant and in sleep disorders. Among the metabolites found in the genus it is highlighted the occurrence of erythrinic alkaloids in several species. In this study, the alkaloid Erythralin was evaluated for anti-tumor activities against human cervical carcinoma cell line (SiHa).

Methods

Cell viability was quantified by the MTT assay and absorbance (570 nm) by an ELISA reader, in each experiment. The apoptotic cells were evaluated using Propidium and Annexin Iodide staining and analyzed by flow cytometry. Nuclear morphological changes were evaluated by fluorescence with DAPI staining and flow cytometry was used to cycle assay.

Results

The alkaloid Erythralin significantly inhibited (p <0.05) the growth of SiHa cells after 24 and 48 hours. The cell viability assay showed that the inhibitory effects of Erythralin were also consistent with the morphological changes observed under light microscopy in a dose-dependent manner. There was also an increase in apoptotic cells in a dose-dependent manner through cytometric analysis. This is the first study that
demonstrated cytotoxic and pro apoptotic effects of Erythralin on SiHa cells. The results also suggest a tendency to stop the cell cycle in the G2-M phase.

Conclusion

Preliminary studies on mechanism reveal that Erythraline induces apoptosis in SiHa cells by arresting the cell cycle at the G2-M phase. However, further evaluations are necessary for the evaluation of its antitumor properties and mechanisms of action. Our results suggest that compound *E. velutina* might be a potential candidate for developing novel anti-cancer drugs in the coming future.
Background / Objectives

Cervical cancer (CC) is the third most common cancers in women worldwide and the fourth major cause of cancer death in the woman in developing countries, remaining a critical public health problem. High-risk human papilloma viruses (HPVs) such as HPV 16, 18, 31 and 33 have been attributed to be the major risk factors for cervical cancer. Platinum based chemotherapy in combination with radiotherapy or surgery is now mainly used to treat CC, but the efficacy is limited especially in advanced-stage disease. Furthermore, these treatments could easily lead to adverse reactions and drug resistance. Therefore, the discovery of new highly selective and efficacy drugs has been the main focus of the research. Thus, the study aimed to investigate, in vitro, the cytotoxic and pro apoptotic effects of leaves and roots fractions from Croton blanchetianus (CB) against human cervical cancer HeLa and SiHa cells.

Methods

Samples were obtained from a crude ethanolic extract after acid-base extraction with chloroform at pH 2 (CBaF from leaves; CBaR from roots) and at pH 9 (CBbF from leaves; CBbR from roots). Phytochemical screening was evaluated by thin layer chromatography using Sulfuric Vanilin, Dragendorff and Natural A Reagent as stain. Cytotoxic activity and apoptosis rates were determined with MTT and Annexin V/PI assays, respectively. Nuclear morphological changes were evaluated by fluorescence with DAPI staining and flow cytometry was used to cycle assay.

Results

According to results, all fractions exhibited terpenoids, alkaloids and flavonoids, except CBbF that showed no flavonoids. All fractions decreased significantly cell viability of HeLa and SiHa in a concentration- and time-dependent manner (p<0,05),
as well as, they induced cellular and nuclear morphological changes, apoptosis and cell cycle arrest (p<0.05).

Conclusion

This is the first study that demonstrated cytotoxic and pro apoptotic effects of Croton blanchetianus on HeLa and SiHa cells. Therefore, Croton blanchetianus appears to be a valuable natural source for the development of agents for the treatment of cervical cancer. However, the present study points to the need for further phytochemical research to isolate the biologically active products of these fractions responsible for the observed activities and to elucidate their action mechanisms.
VULVAR INTRAEPITHELIAL NEOPLASIA – HPV INDUCED PATHOLOGY?

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Background / Objectives

Vulvar intraepithelial neoplasia (VIN) is a premalignant pathology which leads to vulvar carcinoma, the fourth most common gynaecological cancer. Although invasive vulvar cancer rate has remained the same in the last two decades, VIN incidence has doubled. To date, no screening programme exists for early diagnosis of vulvar HSIL, the diagnosis being based on clinical findings and confirmed by biopsy. The aim of this study was to demonstrate if HPV is the carcinogenetic factor incriminated in vulvar cancer.

Methods

During 2011-2016, in the Obstetrics & Gynaecology Clinical Hospital "Panait Sirbu" Bucharest, 20 VIN patients were diagnosed. There were also 12 vulvar cancer which after the confirmation of squamous carcinoma diagnosis, received standard surgical treatment. 9 patients were HPV-PCR tested from biopsy tissue. Surgical treatment was rendered in oncology hospitals with good functional results and no local relapses. HPV infection was present in all 9 biopsy tests, high risk strains 16,18,31,35 and 51 being most commonly found. 6 patients also had positive cervical HPV testing.

Conclusion

Although VIN is not very frequent, because of the appearance on exophytic and endophytic lesions and even vulvar distrophy, the carcinogenetic factor – HPV, should be systematically tested. Early surgical treatment of confirmed vulvar cancer has the best prognostic.
ETIOLOGICAL ROLE OF HUMAN PAPILLOMAVIRUS INFECTION IN THE DEVELOPMENT OF PENILE CANCER

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Background / Objectives

We investigated an etiological role of HPV infection in the development of penile carcinoma.

Methods

Paraffin-embedded tumor samples were collected from 17 patients who had received an operation for penile carcinoma. After DNA extraction from each sample, HPV-DNA test and genotyping were performed using a HPV GenoArray kit (Hybri MaxTM). In addition, localization of HPV was observed by in situ hybridization (ISH) for high-risk HPV-DNA. Furthermore, P16-INK4a and HPV-L1 capsid protein expression were evaluated by immunohistochemistry (IHC).

Results

HPV-DNA was detected in 7 (41%) cases; HPV16 was identified in 5 samples, HPV33 and HPV68 was identified in one case, respectively. ISH analysis demonstrated that high-risk HPV-DNA was localized with punctate staining patterns in the nuclei of tumor cells of all HPV-positive samples. P16-INK4a was moderately to strongly expressed in nuclei and cytoplasm of tumor cells in many of HPV-positive samples, whereas showed the relatively weak or no expressions in HPV-negative ones. On the other hand, HPV-L1 protein expression, which suggested reproductive HPV infection, was not observed in any carcinoma.

Conclusion

The current results suggest that high-risk HPV, especially HPV16 is likely to be a causative agent among an approximate 40% of the Japanese patients with penile
carcinoma, although further studies including a large number of samples are required to reach a more definite conclusion.

References


Cooper K, Herrington CS, Stickland JE, Evans MF, McGee JO. Episomal and


FACTORS ASSOCIATED WITH ABNORMAL ANAL CYTOLOGY OR HR-HPV ANAL INFECTION AMONG HIV POSITIVE MSM

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Background / Objectives

Anal cancer is caused by human papillomavirus (HPV), which can cause changes to the skin around and mucous epithelium inside the anus. Although in the general population is anal cancer a relatively rare neoplasia with incidence between 1 to 2 cases per 100,000 people, estimates of the anal cancer rates among HIV positive men who have sex with men (MSM) range from 70 to 137 cases per 100,000. Anal cytology and HPV detection are important tools for the anal cancer screening. Aim of this study was to compare patient’s characteristics with the anal cytology results.

Methods

In this study HIV positive MSM attending Dermatovenereology Department of Nemocnice Na Bulovce who signed an informed were included. Anorectal cytology and HPV specimens sample were taken from the entire length of the anal canal mucosa using a moistened Dacron swab. After sampling, cells from the swab were washed into a vial with liquid-based cytological medium. In the cytological laboratory, samples were processed to evenly disperse as a thin monolayer of cells while removing background obscuring materials. The presence of anal HPV-DNA was detected by PCR with broad spectrum primers followed by hybridization.

Results

The average age of the 80 HIV positive MSM who agreed to participate in the study was 33.8 years (range 23-57). In our study 65 (81.3%) of the patients were on highly active antiretroviral therapy (HAART) for more than 3 months prior to the specimen collection. Average CD4 cell count of the patients at the time of the study was 671 cells/mm^3 (range 361-1180). HPV-DNA was detected among 71 (88.8%) of the patients. HR-HPV infection was present among 47 (58.8%) patients. Low-grade squamous intraepithelial lesion (LSIL) was detected in 34 (42.5%) patients, normal cytology (NILM) had 23 (28.8%) patients and none of the patients had high-grade squamous intraepithelial lesion (HSIL). HR-HPV anal infection was significantly more common among patients under 34 years of age (73.3% vs. 40.0%; p < 0.01). Presence of abnormal cytology was not associated with patient’s age, HAART or CD4 cell count.

Conclusion
Our preliminary results suggest that anal HR-HPV infection is more common among younger HIV positive MSM. We did not observed association between the presence of HR-HPV infection and HAART or CD4+ cell count over 350. Similarly abnormal cytology among HIV positive MSM in our study was not associated with these factors. Because the results are based on the preliminary data obtained from a small number of patients and none of the patients had CD4 cell count under 350 cells/mm3 they should be interpreted with caution.
HPV genotyping and E6/E7 HPV mRNA expression analyses in anal cytology samples for prevention of HPV-related anal cancer.


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Background / Objectives

Anal cancer incidence is high in populations at high risk, as HIV-infected men who have sex with men, receptive anal intercourse, and high risk sexual behaviour, warranting consideration of early detection approaches. HPV has been shown to be a major cause in the development of anal cancer: persistent infection with high-risk types of HPV causes more than 80% of anal cancers. In the precursor to anal cancer, anal intraepithelial neoplasia (AIN), the prevalence of HPV infection is high. There is no widely accepted procedure guidelines for men with possible exposure to HPV that can lead to dysplasia. The aim of this study is to determine the prevalence of HPV DNA and RNA, to lay the bases for a possible screening strategy for the prevention of anal cancer in high-risk populations.

Methods

We evaluated anal pap test and HPV infection, with both HPV genotyping and mRNA HPV tests, in 129 anal cytology samples collected at the center of the National Surveillance Network of Sexually Infections of Florence in the period 2015-2016, and sent to ISPO for the analyses. The specimens were collected in ThinPrep vials (Hologic). HPV typing was performed by reverse line hybridisation (Ampliquality HPV express AB analitica). Genotype analysis for HPV DNA was available for 52 samples. RNA analysis for E6/E7 HPV mRNA expression was available for 26 samples. The test was performed with Aptima HPV assay (Hologic) with Panther system, that qualitative detects 14 hr- HPV type. 27 samples were also tested for HR-HPV DNA by Cobas 4800 HPV test (Roche).

Results

The prevalence of HPV infection is 78.8% if considering both high risk and low risk HPV types, and 57.7% if considering only hr types. The most prevalence type is HPV16 pos (26.8%), followed by HPV 45 (22%) and HPV 18 (19.5%). Multiple
Infections are frequent: more than 3 types are co-infecting in 25 samples. mRNA HPV was positive in 65.4%. All mRNA-negative samples have negative pap test or non valuable. Of the 45 samples with cytology ≥ ASC-us, 24 were tested for HPV Typing: 79.2% were positive for hr-types; 47.4% of these were HPV16-positive. Cobas HPV test results are concordant (100%) with typing results.

Conclusion

In order to establish a pilot screening programme for anal cancer, it is valuable to determine the prevalence of HPV types and the expression of viral oncogene E6/E7. Anal cytology has been used to predict those at risk of AIN, but the limited sensitivity restricts its usefulness as a potential screening technique. Completing HPV DNA e RNA analyses on all samples, and data from follow up on this group of patients will determine if HPV tests (DNA or RNA) can be applied in a screening contest for anal cancer prevention.
ANAL INTRAEPITHELIAL NEOPLASIA (AIN) AND ANAL SQUAMOUS CELL CARCINOMA (SCC) IN A LARGE URBAN COHORT OF HIV-POSITIVE INDIVIDUALS LIVING IN THE UNITED KINGDOM: A RETROSPECTIVE DATA ANALYSIS

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Background / Objectives

HIV is associated with a 30-fold increased lifetime risk of anal SCC and a 4-fold increase in 5-year mortality. Men who have sex with men (MSM) with HIV are at increased risk of human papillomavirus (HPV) associated cancers including anal SCC. Diagnosis of AIN presents an opportunity to initiate monitoring before SCC development. The characteristics of patients with AIN are poorly understood, as are the factors associated with progression from AIN to SCC. This project aimed to describe the cases of AIN and anal SCC in a large urban cohort of people living with HIV (n=2400) in the UK.

Methods

We identified all cases of AIN and anal SCC in patients attending a single HIV outpatient centre in the UK. We reviewed case notes and histopathology.

Results

23 AIN cases and 28 SCC cases diagnosed 2001-2016: all white MSM. Where documented, 56% were current smokers and 36% ex-smokers. Median age 48 years (range 27-73), nadir CD4 284 cells/mm3 (4-1312), median months since diagnosis 171 (24-361), 100% on antiretroviral therapy (ART). Of the AIN group 16/23 (70%) had previous anorectal sexually transmitted infections (STIs), 15/23 (65%) HPV, 5/23 (22%) gonorrhoea, 4/23 (17%) herpes simplex virus (HSV) and 2/23 (9%) chlamydia. Of the SCC group 24/28 (86%) had documented STI history, 21/24 (88%) with anorectal STIs: 16/21 (76%) HPV, 5/21 (24%) gonorrhoea, 4/21 (19%) chlamydia and 4/21 (19%) HSV. Most AIN patients (83%) presented with an anal lump and the majority (83%) were AIN III. Patients who progressed from AIN to SCC did over 1-9 years, had comparable age (median 52, range 39-72) and nadir CD4 385 cells/mm3( 4-1312) to the broader cohort but were diagnosed with HIV further in the past (210 months,159-226). SCC presenting symptoms were anal lump (75%), pain (21%), and rectal bleeding (17%). 25/28 (89%) had local disease, 3/28 (11%) local nodes with no metastatic disease, 4/28 (14%) had previous AIN. Of those treated at our centre 8/13 (62%) had chemoradiotherapy, 2/13 (15%) had radiotherapy alone, and 3/12 (23%) had surgery. 2/13 (15%) patients needed surgery after unsuccessful chemoradiotherapy. 2/14 (14%) diagnosed with AIN after
successful SCC treatment. 5/28 (18%) patients have died, with 2 deaths attributable to SCC.

Conclusion

AIN and SCC are emerging issues for MSM living with HIV on effective ART. We found that a large proportion of patients had anorectal HPV diagnosed before anal SCC but only a minority had previously diagnosed AIN. Further research is needed to clarify which patients are most at risk of developing SCC and to establish the impact of anal cancer screening on the reduction of anal SCC in this population is urgently needed.
Background / Objectives

The incidence of anal cancer is increasing worldwide, but limited information is available about the prognosis of these tumors. Human papillomavirus (HPV) DNA have been investigated as a prognostic factor in anal cancer. The objective of this study was to retrospectively investigate HPV DNA and clinical data in a series of consecutive cases of invasive anal carcinomas treated in a single institutional service in Brazil. HPV DNA prevalence and genotype distribution were investigated in association with clinicopathological characteristics.

Methods

A group of 81 patients with invasive anal carcinomas was retrospective analyzed for a period of 10 years. Formalin fixed paraffin-embedded samples were tested for HPV detection and genotype distribution by using a SPF-10 Inno Lipa assay. Prevalence ratios were estimated by logistic regression and survival was analyzed by Kaplan Meier and log-rank.

Results

The prevalence of HPV DNA for the whole group was 69%, and it was significantly higher in squamous cell carcinomas (SCC) (88.1%) (OR 9.51 IC 95% 2.96-30.50) and in female patients (78.4%) (OR 3.18 IC 95% 1.19-8.48). Multiple infection was detected in 14.3% of cases and HPV 16, 18 and 33 were the most prevalent genotypes. Overall survival for the group was 44.3%. Survival was significantly higher for men (p = 0.008) and for SCC patients (p = 0.01), and was reduced for patients with distant metastasis (p = 0.01). HPV positive tumors presented with a higher survival, although the difference was not significant (p > 0.05).

Conclusion

The high prevalence of HPV DNA in anal carcinomas was confirmed in this study, however, the presence of HPV DNA did not significantly affect the prognosis of the patients. Prognosis was affected by gender, histological type and distant metastasis.

References
P26-01
HPV DETECTION IN ORAL CAVITY OF ASYMPTOMATIC PEOPLE FROM NORTH ARGENTINA

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Background / Objectives

Infection with HPV is clearly associated with epithelial alteration of genital tract and its oncogenic role is undoubtedly linked to cervical cancer (CC). By contrast, the relevance of the presence of HPV in other sites, like oral cavity, is still poorly studied in major regions of the world with historically high incidence of CC. The aim of our study is to better understand the transmission dynamics of HPV in oral cavity of unvaccinated people from Chaco, a north region of Argentina with a high prevalence of cervical HPV infection and CC. This ongoing research work is feasible thanks to the funds granted by the National Cancer Institute of Argentina.

Methods

Our cross-sectional and observational study shows the results of the first 266 samples of a total of 500 that will be collected by december 2017. Oral rinse/gargle samples were obtained from asymptomatic and sexually active volunteers (women and men). People recruited were from different areas of Resistencia city (Chaco, Argentina), guaranteeing an adequate population heterogeneity and age distribution. A standardized questionnaire was used to interview the participants regarding their clinical history, sexual behavior, cultural habits and socio-economic and living conditions (data not shown). DNA of each sample was extracted and purified by a commercial kit (High Pure PCR Template Preparation Kit, Roche) and the presence of HPV was analyzed by the well known PGMY09/11 general PCR. Positives ones were further studied for HPV genotyping with a commercial kit (HPV Direct-Flow Chip) produced by Master Diagnostica (Vitro Group, Spain).

Results

To date, we have analyzed 266 oral rinses, 118 from men (44.4%; mean age 38 years) and 148 from women (55.6%; mean age 40 years). Six samples (2.3%) were positive for HPV (only in men). There were three cases with a unique HPV detected (HPV-16, -61 and -44/55 respectively); two with multiple genotypes (among them HPV-18, -6, -62/81, -39, -61) and one sample with a non-typifiable HPV type. Regarding the variables possibly associated with HPV infection, it is necessary to finish the samples collection and analysis projected for this work, to conclude properly about them.

Conclusion
HPV infection in the oral mucosa is currently a topic of great interest in many regions of the world. Our study is a contribution to estimate a baseline of oral HPV infection in asymptomatic people. Data collected will be important to analyze, in future studies, the impact of HPV infection in recognized risk groups (People living with VIH, immune-suppressed, oncology patients, etc) and to evaluate the global impact of the current HPV vaccination strategies in Argentina.

References

PREVALENCE OF HPV-16 AND 18 IN PATIENTS WITH ORAL LEUKOPLAKIA: A PRELIMINARY MOLECULAR AND IMUNOHISTOCHEMICAL STUDY FROM A BRAZILIAN COHORT.


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Background / Objectives

Studies have shown that HPV might play a role in the pathogenesis of a portion of oral leukoplakia (OL) cases, nevertheless, highly variable HPV detection rates in this disorder suggests the need for further researches aiming to elucidate which factors might be involved in the development of OL, better explaining the participation of HPV in this process. Therefore, the aim of this study is to evaluate the presence of HPV-16 and HPV-18 DNA, which is the oncogenic genotype of greater relevance, in different biological samples from patients with oral leukoplakia, and the correlation of these factors with sociodemographic and clinicopathologic characteristics and prognosis of OL.

Methods

Fourty patients with OL will compose the study group, and a control group of 40 healthy patients requiring pre-prosthetic oral surgery will be matched to the patients of the study group by sex and age. Tissue, saliva, and blood plasma samples will be obtained from patients of both groups. HPV-16 and HPV-18 DNA detection will be performed in the tissue, saliva, and plasma blood samples from both groups by Real Time PCR (RT-PCR) technique. Furthermore, immunohistochemistry analysis will be performed for p16INK4A in paraffinized tissue samples for evaluating the presence of HPV and the activity of the virus.

Results

Until this moment, a pilot study was performed analyzing the HPV-16 detection among 5 OL patients. Descriptive analysis of clinicopathologic features of these patients demonstrated that most of patients were male (60%), and age ranged from 45 to 66 years old (average = 55.2). Two (20%) were old-aged, 40% were middle-aged, and 40% were young. All patients were current smokers (100%), while none was never or ex-smoker. Most of patients with OL were current drinkers (80%), and 20% were ex-drinkers, while none was never-drinker. 60% of the 5 OL lesions analyzed until this moment displayed some degree of epithelial dysplasia. HPV-16 DNA was not present in none (0%) of fresh tissue and saliva samples from the 5 patients included in the study until now.
Conclusion

No relevant conclusion is possible at this moment. However, further analyses are necessary for better understanding this preliminary study.

References


DNA DAMAGE RESPONSE AS TUMOR SELECTIVE
RADIOSENSITIZATION STRATEGY FOR HPV POSITIVE
AND NEGATIVE HEAD AND NECK CANCERS

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Background / Objectives

Treatment of head and neck squamous cell carcinoma (HNSCC) is characterized by
high local recurrences mainly due to the DNA repair capacity of cancer cells.
Selectively inhibiting these DNA repair mechanisms in combination with radiotherapy
(RT) could increase locoregional tumor control. Moreover, recently we found that
human papillomavirus positive (HPV+) HNSCC cells have differences in their DNA
repair efficiency compared to HPV- cells, suggesting differences in mechanisms of
DNA repair and showing the need for different treatment approaches for HPV+ and
HPV- HNSCC.

Methods

We performed a CRISPR/Cas9-based loss of function screen targeting 36 drugable
genes in DNA damage response (DDR) to discover the most efficient therapeutic
targets that are synthetically lethal in combination with RT for either HPV+, or HPV-
tumors. We validated these results with commercially available drugs (NU7441
(DNA-PK inhibition); ABT-888 (PARP1/2 inhibition); AZD7762 (CHK1/2 inhibition)) by
survival and clonogenic assays. We investigated the repair kinetics by gH2AX and
RAD51 foci formation and the effect on cell cycle by flow cytometry. The therapeutic
efficacy in cell line based and PDX mice models was assessed by tumor growth
delay curves.

Results

Our results revealed 14 hits for HPV+ and 18 hits for HPV- HNSCC, showing the
presence of differences in DDR between HPV+ and HPV- HNSCC. Inhibition of
PARP radiosensitized HPV+ HNSCC cells. This effect was due to p16-mediated
inhibition of homologous recombination repair. This p16-dependent effect also
translated in a slower tumor regrowth in vivo models. CHEK genes were important for
survival of HPV- HNSCC. The majority of the overlapping genes were involved in the
non-homologous end-joining pathway. Validation of these synthetic lethal hits with
DNA-PK inhibitor (NU7441) radiosensitized HPV+ and HPV- HNSCC in vitro and in
vivo.
Conclusion

Our results lead to robust assessment of novel targeted radiosensitizers for HPV+ and HPV- HNSCC.
PREVALENCE AND IMPACT ON SURVIVAL OF HPV AND P16 IN OROPHARYNGEAL CANCER OTHER THAN TONSIL OR BASE OF TONGUE CANCER

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Background / Objectives

Today, most oropharyngeal squamous cell carcinoma (OSCC) is human papillomavirus (HPV) positive and HPV alone or in combination with p16 is reported to be a favorable prognostic factor for OSCC. Patients with tumors at other OSCC sites (OOSCC) are often included in the same treatment and study protocols as patients with tonsillar- and base of tongue SCC, even though the prevalence and clinical significance of HPV infection and the correlation to p16 in OOSCC still is unclear. Since tonsillar and base of tongue SCC cover roughly 90% of all OSCC, there is an obvious risk that there may be a misinterpretation of the results for OOSCC. We have in a previous minor study of 69 patients with OOSCC shown that only a minority (16%) to be HPV positive and 25% to be p16 positive. In addition, there was no complete correlation between HPV status and p16. Furthermore, no impact was seen on clinical outcome for the HPV-positive patients. We are therefore investigating the prevalence and correlation of HPV and p16 in OOSCC and their impact on survival a larger cohort from the Karolinska University Hospital, including patients from 2009-2014. This is of special interest since the International Union Against Cancer (UICC) in their eight edition changed the TNM-classification for p16-positive tumors for the whole OSCC-group.

Methods

All OSCC patients (C10.0–C10.9 and C50.1–C50.8) diagnosed between 2000 and 2014, in the County of Stockholm, Sweden, were included in the study. HPV-DNA was detected by PCR and p16 by immunohistochemistry. The study was conducted according to ethical permissions 2005/431-31/4 and 2005/1330-32 and 2009/1278-31/4 from the Ethical Committee at Karolinska Institutet, Stockholm, Sweden.

Results

Preliminary results: 108 patients have been included so far (2000-2012) so far and of those 22 (20%) had HPV positive tumors. 68 tumors have been tested so far and 11
(16%) were p16 positive. Of the 22 HPV positive tumours, 21 were also tested for p16 and only 8/21 (36%) were positive for both p16 and HPV.

**Conclusion**

Preliminary results show that the of HPV and/or p16 is much lower in OOSCC compared to earlier reports including all OSCC, or tonsillar and base of tongue cancer alone, the correlation between HPV-status and/or p16 in the tumors was not as strong as shown in previous studies including all OSCC or tonsillar SCC. We suggest that HPV/P16-positive OOSCC should not be treated in a similar way to HPV/p16 positive tonsillar and base of tongue cancer until larger studies have clarified the discordance between HPV and p16 overexpression and their clinical impact.
COMPARISON OF ANYPLEX II HPV 28 AND SPF10/DEIA/LiPA25 IN FFPE OROPHARYNGEAL CANCER SAMPLES

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Background / Objectives

Human Papillomavirus (HPV) infection has emerged as a major etiological factor with a prognostic significance in oropharyngeal squamous cell carcinomas (OSCC). The identification of HPV-related tumors in the clinical setting is still mainly based on the p16INK4a overexpression. However, a recently published meta-analysis (Prigge ES, IJC 2017) shows that the combination between p16 overexpression and HPV DNA PCR testing increases the specificity to distinguish HPV-related OSCC. Several techniques focused on HPV DNA detection and genotyping, which have been developed and used for prognosis purposes, could also be used in the near future for treatment decision-making. Here, we compare the results obtained analyzing formalin-fixed paraffin-embedded (FFPE) OSCC tissue blocks using the Anyplex II HPV 28 test and two different extraction methods with the results previously obtained using the SPF10/DEIA/LiPA25 combination for HPV DNA detection and genotyping.

Methods

FFPE samples were selected from an international collection including 1090 OSCCs subjected to histopathological evaluation, HPV-DNA, HPV E6*I mRNA and p16INK4a detection (Castellsagué/Alemany, JNCI, 2016). A subset of 95 FFPE samples were randomly selected from strata specified (40 HPV DNA negative; 40 HPV DNA positive + mRNA or p16 positive; 12 HPV DNA negative+p16 positive; 3 HPV DNA positive + mRNA/p16 negative). DNA extraction for HPV DNA detection (SPF10/DEIA/LiPA25) was performed using a Proteinase K digestion method as previously described. In the present study, samples have been retested using Anyplex II HPV 28 test and two
different DNA extraction methods. Proteinase K digestion method and DNA extraction using the MagCore automatic station were compared. We calculate the agreement between the SPF10/DEIA/LiPA25 and Anyplex II HPV 28 test.

Results

Preliminary data obtained after the analysis of 32 samples show 100% agreement between SPF10/DEIA/LiPA25 and Anyplex II HPV 28 tests. Anyplex II HPV 28 test was positive for 58% and negative for 42% of samples. All HPV positive samples showed the HPV16 type. A complete agreement in HPV detection and genotyping was obtained using Proteinase K digestion method and the MagCore protocol.

Conclusion

Preliminary data have shown a high agreement between SPF10/DEIA/LiPA25 and Anyplex II HPV 28 tests for HPV detection and typing in FFPE oropharyngeal samples. This is an on-going study and complete results will be presented in EUROGIN 2017 congress.

References


THE PREVALENCE OF HPV IN HEAD AND NECK SQUAMOUS CELL CANCER TISSUES OF PATIENTS FROM SOUTH-CENTRAL POLAND


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Background / Objectives

There is growing evidence that human papillomavirus (HPV), in particular HPV16, may be involved in development of some head and neck (HN) squamous cell carcinomas (SCC). However, the prevalence of HPV and its prognostic potential is still the subject of worldwide discussion. Therefore, the aim of our study was to assess the frequency of HPV, its type and prognostic role in patients with HNSCC from south-central Poland.

Methods

The study was carried out in the group of 113 patients with SCC of oral cavity, pharynx or larynx. Experiments were performed using DNA isolated from formalin-fixed paraffin-embedded tumour samples. HPV infection was assessed using nested PCR with PGMY/GP+ primers (according to our best knowledge for the first time in Poland) based on 3-4 experiments for each tissue. Virus type was analyzed by real-time PCR.

Results

DNA was obtained from the material of 109 patients. Based on nested PCR results we qualified 60 (55.0%) of 109 tumours as HPV positive and the infection was confirmed by real-time PCR in 39 (35.8%) of them. The proportion of HPV infection was the highest in oropharyngeal tumours (48.5% of positive cases; 33/68), whereas in oral and hypopharyngeal ones were 20.8% (5/24) and 14.3% (1/7) respectively. None of laryngeal cancer (0/10) had viral infection. HPV16 was the predominant type (82.1%). We also identified 3 cases of HPV35 and 4 of dual infection (HPV35 together with HPV16 or HPV18). The experiments are ongoing and complete data concerning correlation between HPV presence and patients’ survival will be presented during conference.

Conclusion
Some head and neck cancers (mostly within oropharynx) in south-central Poland seem to be HPV-driven, mainly as a result of HPV16 infection, less often because of HPV35 or HPV18.

The study was partially financed by the National Science Centre, Poland project No. 2016/21/N/NZ5/00227.
P27-05
HISTOLOGICAL SUBTYPE OF SQUAMOUS CELL CARCINOMA OF HEAD AND NECK AND THE PRESENCE OF HPV ASSESSED BY THE IMMUNOXESSION OF P16

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Background / Objectives

Head and Neck Squamous Cell Carcinoma (HNSCC) is a malignant disease entity with a high prevalence in the world population (1). Among its major risk factors, there is a persistent infection with Human Papilloma Virus (HPV), which has been related to a better prognosis in patients. HPV infection results in an immunoreactivity of p16 protein that has been used as a marker of the oncogenic lineage by this etiologic agent (3,4). Objective: To analyze epidemiological aspects of patients affected by HNSCC (age, sex and location of the lesion), and relate them to the prevalence of HPV infection. To evaluate the presence of virus stigmas the samples (koilocytes). To correlate histological types and differentiation of HNSCC positive for p16

Methods

A cross-sectional and retrospective study in the electronic archives of the Hospital Universitário Evangélico de Curitiba, in cases with diagnosis of Oropharyngeal SCC, which occurred between January 2005 and December 2015. Slides stained by the HE technique were reviewed and classified histological type of the lesion and and verified of histological stigmas characteristic of HPV (koilocytes). Squamous cell carcinomas (SCC) were classified in keratinizing, no keratinizing and mixed. The paraffin blocks were screened to select the sample areas for the preparation of tissue microarrays (TMAs) in which was performed immunohistochemical study of the p16 protein by avidin-biotin technique. All results and information obtained were tabulated according to data protocol, and then expressed through graphs and tables and statistical analysis was performed by parametric and non-parametric methods, with significance of p <0.05. The project, was approval by the Research Ethics Committee of the Evangelical Society of Paraná.

Results

Of the 51 cases evaluated, 42 were males and 9 females, mean age of 61 years. There was a higher percentage of tumors affecting the larynx (43%), with higher prevalence of keratinized cancers on non keratinized. Koilocytosis was observed in 56.9% of cases, and immunostaining for p16 was 49.02%, predominantly in tumors not keratinized (p = 0.03532).

Conclusion
The present study has demonstrated that the infection prevalence of HPV in HNSCC, through the immunostaining with p16, was present in 49.02% of cases. Toward the epidemiological profile, carcinomas were more common in male individuals with middle ages of 61 years and in the larynx as more often topography. Koilocytosis was found in 29 cases, corresponding to 56.86% of our sample. The immunoreactivity of p16 protein predominated in non-keratinized tumors.

References


Background / Objectives

The signaling pathway of the epidermal growth factor receptor (EGFR) is commonly activated in HNSCC and represents a target for therapy. Among the different anti-EGFR agents, such as tyrosine kinase inhibitors and monoclonal antibodies (mAbs), the reversible inhibitor Cetuximab is the only approved for HNSCC treatment. However, treatment with these reversible tyrosine kinase inhibitors produces objective responses in only a small subset of patients. Despite an initial positive response, these patients often develop or acquire secondary resistance to the inhibitors, leading to relapse after several months. Thus, the resistance to anti-EGFR reversible inhibitors has emerged as an important clinical problem, making irreversible inhibitors studies essential for HNSCC tumors, such as clinical trials with Afatinib and Allitinib. The aim of this study is to evaluate sensitivity and resistance to anti-EGFR targeted drugs in a panel of HNSCC cell lines (HPV positive and negative), and correlate this profile with genetic alterations.

Methods

Five HNSCC HPV(+) cell lines and five HNSCC HPV(-) cell lines were used to test the treatment efficacy of Cetuximab, Afatinib and Allitinib by cell viability assays (MTS). Cells were seeded in 96 wells plates, exposed to increasing doses of each anti-EGFR inhibitors (0 - 2.5 µM) for 72 hours. For the mutation analysis, a panel of primers covering the entire coding extension of TP53, NOTCH1, PIK3CA, FBXW7, HRAS, TP63, CASP8, FAT1, MLL2, RB1, IRF6, NSD1 and EZH2 genes has been customized using AmpliSeq Custom Panel (Life Technologies). The mutational profile of these tumor-related genes was assessed by next-generation sequencing using the Ion Torrent PGM platform.

Results

The results showed that only ten genes (TP53, NOTCH1, PTEN, HRAS, TP63, CASP8, FAT1, MLL2, RB1 and IRF6) had at least one cell line mutated. Several combinations were performed as a panel, where a positive panel was defined as at
least three genes being mutated in the sample, and the result was that 80% of HPV negative cell lines were positive for the panel. Regarding to response profile, all TP53 wild type cell lines were Afatinib sensitive (p=0.033).

Conclusion

The “mutational positive” panel related to HPV negative cell lines corroborate with the literature, where HPV negative tumors are more likely to have these genes mutated than HPV positive tumors. Moreover, TP53 mutational status in HNSCC cell lines may predict Afatinib response, showing its feasibility as a potential biomarker in clinical setting.
THE ROLE OF P16 IN THE METASTASIS PROCESS OF HUMAN PAPILLOMAVIRUS POSITIVE HEAD AND NECK CANCERS

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Background / Objectives

Human papillomavirus (HPV) positive head and neck squamous cell cancers (HNSCC) are often characterized by low tumour (T) and high regional node (N) stages, indicating a high lymphatic metastatic potential. Although the dissemination pattern is different, the haematogenous metastatic rate is the same for HPV-positive and HPV-negative HNSCC. The biological mechanism behind this paradoxal dissemination pattern remains largely unknown.

Methods

We assessed the dissemination pattern in HPV-positive HNSCC combining data of 241 patients with in vitro and in vivo models. More specific, the effect of p16 and HPV on metastasis was assessed by invasion and migration assays. In vivo we focussed on angiogenesis (CD31) and lymphangiogenesis (LYVE-1).

Results

Our study cohort confirmed that HPV-positive patients have significantly lower T stages and higher nodal involvement. HPV-positive cells had significantly lower migration rates and invasion capacities compared to HPV-negative cells. Downregulation of p16 increased migration and invasion capacities. To unravel the metastasis process, we focussed on angiogenesis and lymphangiogenesis, which are indispensable for oxygen and nutrient supply for tumour growth. A negative correlation between HPV and VEGFA was seen in the patient cohort and trend to significance was noted between p16 and VEGFA. Suppression of p16 increased the tumour vascularization. Secondly, HPV-positive tumours showed higher number of lymphatic vessels compared to HPV-negative tumours. P16 suppression in HPV-positive models resulted in lower lymphatic vessel density. This can be related to the α4β1 integrin, an important regulator of lymphangiogenesis, as HPV-negative tumours showed high percentages of this integrin.

Conclusion

These results suggest that p16 inhibits growth and invasiveness through inhibition of angiogenesis but also stimulates local spread by lymphatic vessel formation. This offers therapeutic applications for metastasis in HNSCC by inhibiting
lymphangiogenesis in HPV-positive cancers and angiogenesis in HPV-negative cancers.
P27-08
HUMAN PAPILLOMA VIRUS DNA DETECTION IN FINE-NEEDLE ASPIRATES AS INDICATOR OF HUMAN PAPILLOMA VIRUS-POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: A PROSPECTIVE STUDY.

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Background / Objectives

Human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (SCC) has a better outcome than most head neck squamous cell carcinomas (HNSCCs) and an HPV-positive lymph node metastasis likely has an HPV-positive oropharyngeal SCC origin. Determining HPV-status in cervical lymph nodes by fine-needle aspiration cytology (FNAC) may be useful for diagnosis.

Methods

FNACs from 66 patients with neck masses were prospectively examined for HPV DNA and HPV16 mRNA by a polymerase chain reaction (PCR)-based assay, and the data correlated to diagnosis and HPV-status obtained from histopathological specimens.

Results

Aspirates from 17 of 66 patients, later diagnosed with HPV-positive oropharyngeal SCC, were HPV16 DNA-positive. HPV16 mRNA was detected in all cases with extractable RNA. All remaining FNACs, including 18 branchial cleft cysts, were HPV DNA-negative. HPV DNA status in the aspirates showed perfect concordance with corresponding biopsies.

Conclusion

HPV16 DNA detection in fine-needle aspirations from neck masses is reliable and HPV16 DNA in a metastasis is a strong indicator of an HPV-positive oropharyngeal SCC.
THE COUPLE MANAGEMENT OF HPV INFECTION

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Background / Objectives

Human papilloma virus (HPV) is considered a worldwide public health problem, with 70% of cervical cancers being incriminated to strains 16 & 18 and 90% of genital warts on 6 & 11. With recent tools of molecular biology, we now know there are over 200 genotypes, with very different anatomical site tropism. We aim to demonstrate the implication of HPV in tongue cancer carcinogenesis and the importance of couple diagnosing.

Methods

We present the case of a 27 years old woman who was diagnosed with tongue cancer T2N1M0, in which by PCR reverse hybridization on the biopsy sample HPV 16 strain infection was identified. The patient followed standard surgical and oncological protocol for tongue neoplasm, with favourable outcome, with no signs of local recurrence at two years.

At the same time, she was colposcopically investigated, thus detecting moderate cervical dysplasia (CIN 2), while cervical HPV typing identified the same 16 strain infection. The cervical lesion was surgically treated by diathermal loop electroresection, and the HPV retesting at 3 months post-intervention was negative. The patient received the anti-HPV tetravalent vaccine, using the complete protocol.

The partner, age 41, was diagnosed in a dermato-venerology service with penile warts. HPV genotyping identified the strains 16, 31 and 40 as positive. Penile lesions were cauterized and the patient was vaccinated anti-HPV on request with complete protocol without local recurrence at two years.

Conclusion

Interdisciplinary collaboration, consisting in a medical team of otorhinolaryngologists, oromaxilofacial surgeons, gynaecologists, urologists and dermatologists, contributed to the optimal management of this case of couple HPV infection.

The detection of one or more strains of HPV in the genital or otolaryngology area in one of the partners of a couple requires a complete medical history and physical...
investigations for the detection of all possible HPV-induced lesions regardless of their location in both partners.
Background / Objectives

Patients with oropharyngeal squamous cell carcinoma (OpSCC) have significantly improved overall survival (OS) compared to matched HPV negative control groups. This observation has led to several ongoing clinical trials evaluating deintensification treatment strategies in HPV positive disease. Current guidance from UK national and professional organisations recommend p16 immunohistochemistry (IHC) as a minimum test which should ideally be followed by HPV-specific testing with DNA in situ hybridisation (ISH) in p16 positive cases.1,2 However, most UK diagnostic and treatment centres limit HPV testing to p16 IHC alone. Furthermore, several clinical trial protocols randomise patients according to p16 status alone without necessitating HPV specific testing. The objectives of this study were to:

1. Determine the prevalence of p16 positive tumours lacking high-risk HPV DNA by ISH (p16+/DNA ISH-) in OpSCC.

2. Compare OS of patients with p16 positive tumours demonstrating high-risk HPV DNA ISH positivity (p16+/DNA ISH+), p16+/DNA ISH- and p16 negative (p16-) OpSCC.

3. Determine whether p16+/DNA ISH- can further be classified using RNA ISH.

Methods

Consecutive OpSCC cases from two large UK treatment centres were tested for p16 IHC. High-risk HPV DNA ISH was undertaken on all p16 positive tumours. RNA ISH was performed on a subset of p16+/DNA ISH- cases. OS was determined from patient records and evaluated using SPSS software.

Results

There were 347 patients included in this study. The prevalence of p16+/DNA ISH- was 11.8%. Patients with p16+/ISH- OpSCC had poorer OS compared with p16+/DNA ISH+ tumours (mean OS 45.1 vs 53.2 months, p=0.001), but improved OS compared with p16- tumours (mean OS 45.1 vs 33.7 months, p=0.023). Twenty-four p16+/ISH- samples were available for RNA ISH testing, of which 15 were finally classified as HPV positive and 9 as HPV negative.

Conclusion
Patients with p16+/DNA ISH- OpSCC have poorer OS compared to those with p16+/DNA ISH+ tumours, but improved OS compared with p16- tumours. Therefore, for purposes of clinical trial stratification and prognostication, we recommend HPV DNA ISH testing on all p16+ cases. RNA ISH is a suitable test for resolving the HPV status in p16+/DNA ISH- tumours.

References

1. https://www.nice.org.uk/guidance/NG36/chapter/Recommendations#hpvrelated-disease

FREQUENCY AND CLINICAL OUTCOME OF HPV-DRIVEN OROPHARYNGEAL CARCINOMA IN NORTH-EAST ITALY

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Background / Objectives

HPV-driven oropharyngeal carcinoma (OPSCC) is on the rise in many European countries. Our objective is the assessment of frequency over time of HPV-driven OPSCC in our area and the clinical outcome in comparison to HPV-unrelated cases.

Methods

Fresh/frozen or formalin-fixed paraffin-embedded tumor specimens obtained at OPSCC diagnosis from consecutive patients (period evaluated 2003-2016) were analyzed by PCR with MY09/MY11 primers and restriction fragment length polymorphism analysis for search and typing of HPV-DNA sequences; HPV16 viral load (E6 copies/cell) was determined by real-time PCR; p16 expression was evaluated by immunohistochemistry. Presence of HPV-DNA, HPV16 viral load >1 E6 copy/cell, and p16 overexpression defined an OPSCC as HPV-driven. Frequency of HPV-driven OPSCC was calculated overall and by time periods (2003-2007; 2008-2012; 2013-2016). Overall Survival (OS) and Progression Free Survival (PFS) were calculated by Kaplan-Meier method and Cox-regression and compared among patients with HPV-driven and HPV-unrelated OPSCC.

Results

Overall, 101 cases of newly diagnosed OPSCC were included; data on 63 of them have been previously published (1-3). HPV-DNA sequences were detected in 31 of them; HPV16 was present in 29, and HPV58 and HPV33 in 1 case each. Up to now, the causal role of HPV has been proven in 28 cases (26 HPV16, 1 HPV58 and 1 HPV33). The prevalence of HPV-driven OPSCC in the three time periods was 17.4% (4/23), 25.6% (10/39) and 35.9% (14/39), respectively (P=.109). Patients with HPV-driven tumors had both improved OS (HR = 0.25, 95% CI = 0.10 to 0.62; P=.003) and PFS (HR = 0.23, 95% CI = 0.10 to 0.55; P=.001).

Conclusion

The frequency of HPV-driven OPSCC is on the rise also in North-East Italy, an area with known low prevalence. Our data confirm the good prognosis of patients with HPV-driven OPSCC.
References


PREVALENCE OF HPV IN BRANCHIAL CLEFT CYSTS AND THE USE OF HPV IN DIAGNOSIS OF CYSTIC LESIONS OF THE NECK

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Background / Objectives

The difficulty to distinguish a branchial cleft cyst from a cystic metastasis is well known. Branchial cleft cyst is a benign condition which is treated by surgery with relatively low morbidity. Cancer of unknown primary has formerly been treated with neck dissection and then radiotherapy. We, like many head and neck cancer centers in the world, has in recent years relied on the cytological diagnosis of SCC (which can be hard) in combination with HPV analysis, and if the metastasis is HPV positive it is assumed that the primary tumor is in the base of tongue or the tonsils. Then the patient receives radiotherapy. The problem is that this shift in diagnostic and treatment has occurred, even internationally, without anyone really examined whether HPV can also occur in branchial cleft cysts. The risk is that if we rely on HPV assay as a diagnostic tool for cancer, even if branchial cleft cysts are found to be harboring HPV, patients with HPV positive branchial cleft cyst can receive unnecessary cancer treatment.

In a previous prospective study of FNAC in neck masses we found 18 branchial cysts which all were HPV-negative.

Methods

All patients over 18 years who underwent surgery for branchial cleft cysts from 2005 to 2015, about 400 pc, is included. HPV assay is performed with Multiplex Luminex HPV PCR method in histological sections from paraffin-embedded material from 2005-2015 from the cyst surgically removed.

Results

12 branchial cleft cysts have so far been analyzed, and they have all been HPV-negative.

Conclusion

Reliable methods for diagnosis of cystic lesions the neck is essential for proper diagnosis and treatment. There is, so far, not any published articles regarding HPV in branchial cleft cysts.

References
EXPRESSED HPV INTEGRATION EVENTS IN HEAD AND NECK CANCER: WHERE THEY OCCUR AND THEIR EFFECT ON SURVIVAL AND MOLECULAR SIGNATURES

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Background / Objectives

The incidence of human papillomavirus (HPV)-related oropharyngeal cancer has risen to epidemic levels in the United States. One common molecular event during HPV-related oncogenesis is full or partial integration of the HPV genome into the host genome. Expression of the integrated HPV early genes has clinically-relevant effects. However, the specific nature of where these integration events occur, their effect on patient survival, and the extent to which the switch to integrated HPV integration affects differentiation, the host immune response, and DNA copy number changes is unknown. Previously, our group characterized two HPV-related tumor subtypes, finding that they differed by HPV integration status, immune response, keratinization, EMT signatures, type and number of DNA copy number alternations, and PIK3CA mutations. Interestingly, many of the differences could be explained by HPV integration.

Methods

In this study, we identified and characterized expressed HPV integration events from 84 HPV-positive oral cavity and oropharyngeal tumors (18 from the University of Michigan Hospital (UMH) and 66 from The Cancer Genome Atlas (TCGA)). Flash frozen tumor tissue was collected at UMH for 36 oral cavity and oropharyngeal cases, and H&E slides were assessed for degree of cellularity (minimum 70%) and necrosis (less than 10%). Upon mRNA-seq using 100nt paired-end reads on an Illumina HiSeq, half (18/36) were identified as HPV-positive. HPV integration events were detected using RNA-seq data, captured by HPV-host fusion transcripts. Virus-seq was used to detect HPV-host fusion events, the edgeR R package was used to determine differential expression, and a Cox proportional hazards model was used for survival analysis.

Results

We found 320 virus-host integration breakpoints in 51 (61%) of the 84 samples. Integration events were strongly overrepresented near known head and neck, lung, and urogenital cancer genes, with five recurrent genes (including PD-L1). They were enriched in certain classes of repetitive regions, and a significant number of genes harboring an integration were found to interact with Tp63, ETS, or FOX1A. Patients
with no detected integration had significantly better survival than those with a detected integration and HPV-negative patients.

**Conclusion**

Our results suggest that in HPV-related tumors of the upper aerodigestive tract, there is strong natural selection for cells with expressed HPV integration events in/near key oncogenes and tumor suppressors. The survival benefit of those patients having no expressed integration event provides a candidate cohort for de-escalated therapy.

**References**

E6 PROTEINS OF ALPHA AND BETA CUTANEOUS HPV TYPES DIFFER IN THEIR ABILITY TO POTENTIATE WNT SIGNALING

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Background / Objectives

HPV types which belong to the beta-PV genus have been implicated in the development of non-melanoma skin cancer (NMSC). Our recent studies found that the E6 protein of HPV16, a mucosal high risk HPV type from the alpha genus, is capable to cooperate with the ubiquitin ligase E6AP to enhance or stimulate Wnt/beta-catenin/TCF transcription. In the present study we investigated the transcriptional activities of E6 proteins of diverse HPV types that infect the skin, both from the beta and alpha HPV genus.

Methods

Luciferase reporter gene assays, Western blots, immunoprecipitation and immunofluorescence analyses

Results

Using reporter gene assays, we show that similar to 16E6, E6 of HPV10, an alpha HPV type which is prevalent in skin warts, is capable to efficiently augment as well as stimulate Wnt/beta-catenin/TCF transcription. Western blot and immunofluorescence analyses indicated that 10E6 also elevated efficiently the expression levels of beta-catenin and promoted its nuclear accumulation. E6 proteins of the beta HPV genus including HPV 8, 24, 38 and 49, exhibited lower activities in enhancement or stimulation of beta-catenin/TCF transcription, as well as reduced ability to stabilize β-catenin. The difference in levels of activity between the alpha and beta HPV E6 proteins correlated with the ability of the proteins to interact with E6AP.

Conclusion

This study revealed a role for E6 proteins of diverse skin associated HPV types in potentiating Wnt/beta-catenin/TCF signaling irrespective with their carcinogenic potential.
P29-01
LOW VACCINE COVERAGE BUT NEAR EXTINCTION OF HPV 6 AND GENITAL WARTS IN YOUNG WOMEN IN WOLFSBURG, GERMANY

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Background / Objectives

It has been shown that vaccination of HPV naive women against HPV 6/11 protects sufficiently from genital warts and may even lead to protection of non-vaccinated men and women in the same population. However it is uncertain what level of vaccine coverage is needed for such cohort effects.

Methods

WOLVES (Wolfsburg HPV epidemiological study) invited all women born 1983/84 and 1988/89 with a first residency in Wolfsburg to participate in 2009/10. Participants born 1988/89 were followed with annual examinations from 2009/10 till 2014/15. Women born 1993/94 were first invited 2014/15 and are followed with annual visits. HPV-testing is based on LR and HR-HC2 followed by HPV genotyping with SPF-10 PCR of all HC2 positive and 10% of HC2 negative samples.

Results

Between Oct 2009 and Dec 2015, 2,360 women were recruited. The HPV vaccination coverage rate rose from 6.1% among 26 years old women in 2010 to 18.4% in 2015 while the corresponding rates for 21 years old women increased from 23.7 to 48.2%. Simultaneously the life risk to suffer from at least one episode of genital warts before age 27 dropped from 4.7% in 2010 to 2.5% in 2015 while the life risk before age 22 declined from 1.8% to 0.4%. This trend of disappearance of genital warts was underlined by a decline in the prevalence of HPV 6 from 2.1% to 0.2% among 26 years old women between 2010 and 2015 and from 2.0% to 0.0% among 21 years old women.

Conclusion

The unexpected significant drop in genital warts and HPV 6 prevalence in a population with suboptimal HPV vaccine coverage is reassuring. Obviously the transmission of HPV as the causal agent of most genital warts is strongly inhibited even in populations with low vaccination coverage.
ACCURATE AND SPECIFIC DETECTION OF 13 GENOTYPES ASSOCIATED WITH SEXUALLY TRANSMITTED DISEASE BY PNA MEDIATED REAL TIME PCR

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Background / Objectives

Sexually transmitted diseases (STD), also referred to as venereal diseases (VD) are diseases that can be spread by sexual activity or sexual contactless infection such as transmission via infected blood. Some of STD pathogens such as *Gardnerella vaginalis* (GV) show no symptoms in men but has the ability to infect women. Other pathogens such as *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) may appear chronic pain, itching, small fluid-filled blisters and painful sexual intercourse or even infertility in women and it can be prescribed antibiotics. *Neisseria gonorrhoea* (NG) causes symptoms of cervicitis, urethritis and others in women and men both. NG or CT can be transmitted from a pregnant mother to unborn child. Therefore, detection of STD pathogens is essential for accurate treatment because each type of STD pathogens requires different prescription of antibiotics.

Methods

We have developed a highly sensitive and simple real-time PCR method to detect 13 types of STD pathogen from DNA in patient’s specimens such as cervical swab and urine both for female and male. This method (PANA RealTyper™) uses specific peptide nucleic acid (PNA) probe conjugated with a fluorescent dye and a quencher. These PNA probes are designed to their specific target sequences, which results in each having unique Tm value.

Results

Test was performed to obtain 40 cervical swab samples and 35 urine samples. DNA was extracted from 40 cervical swab samples and 35 urine samples. PANA RealTyper™ STD analysis showed a concordance rate of 92% in 69 samples of 75 samples. Mismatched 6 samples were confirmed via sequencing, it showed that the result of PANA RealTyper™ STD matched sequencing PCR. 40 of 85 clinical specimens were infected samples with two or more STD pathogens.

Conclusion

RealTyper™ STD was able to detect multiple pathogen targets at the same time. PANA RealTyper™ STD is able to detect 13 types of different pathogens with detection limits as low as 5x10^1 copies/rxn using standard materials. It provides genotyping of a total 13 types (*Chlamydia trachomatis, Trichomonas vaginalis,*...
Ureaplasma pavum, Ureaplasma urealyticum, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Haemophilus ducreyi, Herpes Simplex Virus 1, 2, Gardnerella Vaginalis, Candida albicans, Treponema pallidum) by real-time PCR.
HPV infection among HIV-positive men: A four year revised experience of an diagnosis Laboratory.

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**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, 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 as well as anal, penile, 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 by vaccination and information, a priority.

The authors present a 4 years revised casuistic as a reference laboratory center in sexually transmitted infection diseases diagnosis.

**Methods**

Male samples were tested by HPV-molecular and conventional-cytology methods. HPV molecular methods used where: Hybrid Capture 2 (hc2, Digene) ; Clart human papillomavirus 2 (Genomica) and PapilloCheck . The cytological results were registered with comprehensive classification system, multi-axial nomenclature SNOMED. The diagnosis of “classic” sexually transmitted infections (STIs) as Herpes Simplex virus 1 and 2, Syphilis, Gonorrhea, Chlamydia trachomatis, Ureaplasma and Mycoplasma infections statistics were used for data analysis; the Fisher exact test was employed to assess the association between categorical variables. P-values (2-sided test) less than 0.05 were considered significant.

**Conclusion**
The results obtained for the incidence of most frequent HPV genotypes in men and MSM are in agreement with several studies. Type 16 was consistently found among the most common; however, other types were also reported (types 6, 11, 18, 31, 33, 42, 52, 53, 54, 59, and 84) but a shift possibility can occur with universalization of the vaccine. HPV infection appears to occur early in MSM. The majority of MSM followed in Proctology consult first diagnosed for anal or perianal condyloma was offered starting HPV vaccination. It will be an interesting development of this work, following up some of these patients and document relapsing and the HPV genotypes evolved after complete vaccination schemes.
ESTIMATING THE EPIDEMIOLOGICAL IMPACT AND COST EFFECTIVENESS OF THE NEW NONAVALENT HPV VACCINE IN SPAIN

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Background / Objectives

Human papillomavirus (HPV) is one of the most frequent sexually transmitted infection and one of the main causes of infection-related cancer, accounting for 5% of the total burden of human cancer worldwide. Since 2007, two vaccines were available in Spain: the quadrivalent HPV vaccine (HPV4), that contains types 6/11/16/18 and the bivalent HPV vaccine (HPV2), that contains types 16/18. It is estimated that 6/11/16/18 are responsible of 47% of precancerous anogenital lesions due to HPV, and 79% of cancers related to HPV. A nonavalent vaccine (HPV9) containing HPV types 6/11/16/18/31/33/45/52/58 has been developed. According to epidemiological data, the 9 types are responsible of 82% precancerous lesions and 90% of cancers related to HPV.

The aim of this project is to assess the epidemiological impact and the cost effectiveness of 9vVPH in Spain.

Methods

We adapted to the Spanish setting an integrated HPV disease transmission model that accounts for herd protection effects and with a 100-year time horizon.

Results

The model shows further reductions in the incidence and mortality of diseases related to HPV16/18/31/33/45/52/58 types over the analyzed time horizon with HPV9 vaccination when compared to HPV4 or HPV2 in both girls-only and universal vaccination scenarios. 9vVPH would avoid additional 161,485 CIN1 cases, 112,393 CIN2/3 cases, and 15,380 cervical cancer cases compared to HPV4 and HPV2 in a girls only vaccination scenario. With regards to genital warts, 1,466,379 and 1,395,111 cases, in females and males respectively, would be prevented by HPV9 versus HPV2 in a girls only vaccination and 1,629,959 cases in females and 2,027,372 cases in males, in a universal vaccination setting.

Girls-only vaccination strategy of a 12 years old cohort with HPV9 was found to be cost effective compared to HPV4 (ICER of €14,000/QALY) and a dominant strategy compared to bivalent HPV vaccine (HPV2). In a universal vaccination scenario,
HPV9 is cost-effective compared to HPV2 (ICER of €3,716/QALY) and is cost-effective vs. HPV4 Girls if HPV9 price does not exceed €121 per dose.

**Conclusion**

A significant reduction in the HPV-related disease is expected after HPV9 introduction. In addition, HPV9 vaccination is cost-effective when compared to the current HPV4 and HPV2 vaccination programs. Moreover, the inclusion of boys in the HPV9 vaccination program could potentially further reduces the burden of HPV-related diseases in both genders in Spain.
P32-02
GARDASIL9: ACCELERATED REDUCTION IN THE INCIDENCE AND COSTS OF HPV-RELATED PRECANCEROUS LESIONS AND CANCERS

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Background / Objectives

Gardasil9 – protecting against HPV 6, 11, 16, 18, 31, 33, 45, 52 & 58 - is available and indicated in males and females to protect against

- Premalignant lesions and cancers affecting the cervix, vulva, vagina and anus caused by vaccine;

- Genital warts (Condyloma acuminata) caused by specific HPV types (1).

Compared to Gardasil and Cervarix, Gardasil9 improves the protection against precancerous lesions and cervical, vulvar, vaginal and anal cancers (2) implying that Gardasil9 lead to an accelerated reduction in the incidence of precancerous lesions in the cervix, vulva and vagina and anus and in the incidence of cervical, vulvar, vaginal and anal cancers. Consequently, the future saved costs of treatment of precancerous lesions and costs of treatment of cancer will be reduced even more.

In this study, the extra costs saved in Denmark using Gardasil9, compared to Cervarix and Gardasil, in the Danish HPV-vaccination programme targeted girls will be estimated.

Methods

The analyses are based on previous published model simulations and updated unit cost estimates (3). In addition, the following limitations and assumptions are made:

- It is assumed that Gardasil and Cervarix has the same relative protection against CIN2+ (cervical intra-epithelial neoplasia), cervical, vulvar and vaginal cancers;

- Since Gardasil9’s extra protective effect against anal cancer is little, and since unvaccinated men (and ignoring the herd immunity protection) also are diagnosed with anal cancer, this extra effect is ignored in the calculations; and

- Since no Danish unit cost estimates for the precancerous lesions in the vulva or vagina and anus (VIN 2/3, VaIN 2/3 and AIN 2/3) are published/available, Gardasil9’s extra protective effect against VIN 2/3, VaIN 2/3 and AIN 2/3 is also ignored

Results
Compared to Cervarix and Gardasil, the extra costs saved given Gardasil9 vaccination is estimated to 3.2 mill. € (PV: present value) per vaccinated cohort. Especially the additional reduced incidence of CIN2+ and cervical cancer lead to sizeable extra costs saved – 2.5 mill. € (PV) and 0.65 mill. € (PV), respectively.

In addition, Gardasil and Gardasil9’s protection genital warts lead to extra saved treatment costs compared to Cervarix.

**Conclusion**

In a Danish setting, a Gardasil9 vaccination programme will lead to an increased reduction in the incidence and costs of HPV-related precancerous lesions and cancers.

**References**

COST-EFFECTIVENESS OF CERVICAL CANCER SCREENING IN EUROPE – A SYSTEMATIC REVIEW WITH SPECIFIC INTEREST ON RISK-ADAPTED STRATEGIES

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Background / Objectives

In Europe, cervical cancer screening varies substantially regarding age at screening start and end, frequency, type of primary test and follow-up algorithm for screen-positive women. Risk-based screening and follow-up strategies may have the potential to improve both, the benefit-harm balance of the screening program and its cost-effectiveness. We systematically reviewed current evidence on long-term effectiveness and cost-effectiveness of cervical cancer screening in Europe with specific interest on risk-adapted strategies.

Methods

Relevant databases (Medline/Embase/Cochrane Library/CRD/EconLit) were systematically searched for decision-analytic studies evaluating the cost-effectiveness of cervical cancer screening strategies in Europe. Study characteristics and results including the incremental cost-effectiveness ratios (ICER) in cost per quality-adjusted life year (QALY) or life year gained (LYG) were extracted into standardized evidence tables. Economic results were converted to 2015 Euros.

Results

Fourteen studies were included, comprising eleven analyses for countries with population-based organized screening, one for an opportunistic screening, and two for countries where, depending on the region, the screening is organized or opportunistic. Three studies evaluated screening for multiple European countries.

HPV-based screening was reported to be more effective in terms of patient-relevant outcomes compared to cytology alone in both non-vaccinated and vaccinated
women. Overall, HPV-based screening strategies were considered to be cost-effective at a willingness-to-pay threshold of 50,000 Euro/QALY or LYG conditional on screening intervals of at least three years in non-vaccinated women and at least five years in vaccinated women. Most studies recommended starting screening at age 25 with HPV-based screening at age 30 years or older. HPV screening was mostly accompanied with a triage test for HPV-positive women. The upper age limit for screening varied with most studies ending screening at age 65 years.

**Conclusion**

In conclusion, the evidence from decision-analytic modeling studies suggests that HPV-based screening is more effective compared to cytology alone, and can be considered cost-effective at screening intervals of at least 3 years in non-vaccinated and at least 5 years in vaccinated women. Current risk-tailored screening programs are based on restrictions to a specific age and using triage or follow-up tests for HPV-positive women before referring to colposcopy directed biopsy. In future research, predictive biomarker for risk-based management of screen-positives should be considered.
A SURVEY OF HPV KNOWLEDGE AND ATTITUDES TOWARD HPV VACCINATION AMONG THE MIDDLE SCHOOL STUDENTS AND THEIR PARENTS IN CHINA*

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Background / Objectives

Cervical cancer is one of the most common cancers afflicting women worldwide. The wide disparities in the incidence and mortality of cervical cancer are mainly attributable to irregular education and economic development in China. We assessed the knowledge of HPV and attitudes towards HPV vaccination among middle school students and their parents in Changzhi city of north China from June 2015 to March 2017, in order to provide an evidence for the development of an effective national HPV vaccination program.

Methods

We selected 6 middle schools from urban and rural area in Changzhi city of north China. 2 first-grade classes were randomly choose from each school as the intervention group (given education related to knowledge of cervical cancer and HPV vaccination), and other first-grade classes in the same schools as the control group. The health education was conducted by the qualified teacher for intervention group. The questionnaire related the knowledge on cervical cancer was conducted before and after class. The same test was performed in the control group. The two groups were followed up to evaluate the effect of education in 2016 and 2017 respectively. Their parents of the students were required to accept the education of cervical cancer and HPV vaccination, and complete the questionnaire before and after the lecture.

Results

1241 students, aged from 11-15 years, from 6 middle schools attended the study in the baseline survey, 568 in intervention group and 673 in control group, respectively. We found only 34.89% of participates heard of cervical cancer, 10.87% heard of HPV, 7.17% heard of HPV vaccine. After intervention, there were a significantly improvement the awareness about cervical cancer and HPV vaccine ($P<0.05$). 91.02% of participates would like to HPV vaccine ($P<0.01$). There were a significant difference between intervention and control group ($P<0.01$). Comparing the follow-up results of survey in 2016 and 2017, we found there still were a significant with the awareness between intervention and control group ($P<0.05$). In the baseline, we found that 29.87% of 233 parents heard of the HPV, and 20.34% heard of the HPV vaccine in the survey. Also, there were a significantly improvement the awareness about cervical cancer and HPV vaccine ($P<0.05$) after the health education.

Conclusion
The level of knowledge on cervical cancer and HPV vaccine was lower in the subjects. We should strengthen public and middle school students' education with regard to cervical cancer and HPV vaccination to support increased uptake of cervical cancer prevention and control in China. Also, it is necessary to shorten the interval between health educations in order to improve the effect of it.

References

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HPV – WHAT DO THEY KNOW: EVALUATION OF MEDICINE STUDENTS AND HEALTH PROFESSIONALS – VALENÇA – RIO DE JANEIRO/BRAZIL

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Background / Objectives

One of the most prevalent sexually transmitted diseases affecting the population is Human Papillomavirus (HPV), which is one of the most common infections among young and sexually active individuals, in which 75-80% of the population has been, is or will be infected during his life. The lack of information about the Human Papilloma Virus, the signs and symptoms of the infection, its relation to cervical cancer and the forms of transmission, contributes to the female sex being more exposed to this disease. Thus, there is a need to research the understanding of the students and health professionals of an educational institution regarding HPV, since information is the main basis of health prevention. Objective: To analyze the knowledge of medical and non-medical professionals, medical students and students of other courses regarding HPV (Human Papilloma virus).

Methods

We interviewed 269 people in the city of Valença - RJ, with an identification questionnaire and questions about HPV. Among those interviewed are: doctors, medical students, non-medical professionals and students from other courses of the Dom André Arcoverde Foundation - FAA.

Results

The results were divided into 4 variables, such as: interviewee profile, knowledge of health professionals, high school students and non-medical professionals about HPV, relationship with uterine cancer and respect for the vaccine, questioning about acceptance of the vaccine against HPV.

Conclusion

The results of the study indicate that the majority of health interviewees have knowledge about the Human Papilloma virus, its etiology, as well as its form of transmission and prevention. On the other hand, it is noticed that there is still a lack of knowledge on the part of the staffs and students of other courses.

References


Well-Child Visits and HPV Vaccination in Preteenagers Aged 11-12 Years during 2007-2015 in the United States

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Background / Objectives

Well-child visits (WV) provide the best opportunities for vaccinations in the US. The Advisory Committee on Immunization Practices (ACIP) recommends vaccination of 11-12 year old females and males with HPV (Human Papillomavirus) vaccine. This study aims to estimate the % of preteenagers aged 11-12 years who had WV and the % received the first dose of HPV vaccine (HPV1) from 2007 to 2015 and to estimate, for those preteenagers who did not receive HPV1, the % of them had a WV in the future.

Methods

This was a retrospective database (MarketScan®) cohort study. Eligible subjects were 11-12 year old females and males who had continuous enrollment since January 1, 2007 or January 1 of the year they turned 9 years old and did not have HPV vaccine previously. Females were excluded if they had a medical claim related to pregnancy, delivery, cervical cancer, or hysterectomy. Percentages of WV and HPV1, overall and during WV, were estimated.

Results

There were a total of 1,922,372 eligible subjects; 58.8% 11 years old vs. 41.2% 12 years old and 78.2% females vs. 21.8% males. From 2007-2015, 51.3% of 11 year olds and 53.4% of 12 year olds had WV; 9.8% of 11 year olds and 14.0% of 12 year olds initiated HPV1. Among those who initiated HPV1, approximately 53% were during WV for both 11 and 12 year olds. For those who did not receive HPV1 at 11-12 years old, less likely they would have a WV in the future, ranging from 36.6% at 12-13 years old to 5.2% at 19-20 years old.

Conclusion

This analysis suggests that WV at 11-12 years of age provide the best opportunity to maximize the potential of the HPV vaccination program in the US.
ACCEPTABILITY AND SHORT-TERM EFFECTS OF AN HPV VACCINATION INTERVENTION FOR YOUNG GAY AND BISEXUAL MEN IN THE UNITED STATES

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Background / Objectives

Gay and bisexual men have high rates of human papillomavirus (HPV) infection and HPV-related disease, such as anal cancer. Despite these existing disparities, no known interventions have been developed to increase HPV vaccination among young gay and bisexual men (YGBM) in the United States. We developed and pilot tested an online intervention, Outsmart HPV, to promote HPV vaccination among YGBM.

Methods

In 2016, we used social media to recruit a national sample of YGBM ages 18-25 in the United States who had not received any doses of HPV vaccine (n=150). Participants were randomized to receive either standard information about HPV vaccination (control group) or population-targeted, individually-tailored content about HPV vaccination (intervention group). Participants in both study arms completed a baseline survey before viewing their study materials and a follow-up survey immediately afterwards. We used multiple linear regression to assess between-group differences in attitudes and beliefs about HPV vaccination.

Results

Most participants were ages 22-25 (59%), gay (83%), non-Hispanic white (57%), and not married or living with a partner (80%). There were no differences in HPV vaccination attitudes and beliefs between study arms at baseline. Compared to participants in the control group, participants in the intervention group reported on their follow-up surveys: greater perception of increased risk for anal cancer among men who have sex with men compared to other men (b=0.30), fewer perceived harms of HPV vaccine (b=0.34), and greater self-efficacy to get HPV vaccine (b=0.18) (all p<0.05). Results also suggest a trend toward higher intent to get HPV vaccine among participants in the intervention group compared to those in the control group (b=0.21, p=0.09). Participants in the intervention group reported high levels of acceptability and satisfaction with their viewed study materials about HPV vaccination (all means >4.40 on a 5-point scale). Further, participants in the intervention group more strongly endorsed that their materials were easy to understand than did the control group (means: 4.72 vs. 4.42, p<0.05).

Conclusion
Findings from this pilot randomized controlled trial provide preliminary support for an online HPV vaccination intervention being highly acceptable to YGBM and improving their attitudes and beliefs about HPV vaccination. Taken together, these results suggest that Outsmart HPV may be a promising tool for promoting HPV vaccination among YGBM. An important next step is to determine the efficacy of the intervention on increasing HPV vaccine uptake among this population.
Background / Objectives

In the U.S. uptake of HPV vaccine remains significantly behind the Healthy People 2020 goal of 80% series completion. While some countries have implemented very successful HPV immunization programs, others have encountered significant political, policy, and logistical barriers. In the U.S., the policy, implementation, and adoption of HPV vaccine has been particularly complicated. As with many other medical innovations, diffusion and adoption is not always rapid and depends on a variety of social and cultural factors, as well as the nature of the innovation itself. Research indicates there is a great deal of 1) confusion and uncertainty about HPV vaccine and 2) concomitant misinformation about the HPV vaccine, who it is meant for, and the conditions under which it is maximally effective. The objective of our study was to develop and evaluate a web-based approach to encourage HPV vaccination in New Mexico, an ethnically diverse U.S. state.

Methods

With funding from National Institute of Allergy and Infectious Diseases, our team conducted a project to systematically develop a set of web-based tools to prompt the informed adoption of HPV vaccination. We used Diffusion of Innovations Theory and related research on Informed Decision Making to guide the iterative development of a website for parents of young female adolescents.

Results

Our presentation will review the website (GoHealthyGirls.org) and present development and preliminary efficacy data from the study. Subsequent funding from the Patient Centered Outcomes Research Institute (PCORI) and the National Cancer Institute (NCI) has provided the opportunity to translate the GoHealthyGirls website to a mobile device responsive format (mobile web app: Vacteens) for parents and girls and to develop a parallel web app intervention for young adolescent boys and their parents. Both new projects will involve large cluster randomized controlled efficacy trials with parents and their adolescent children in New Mexico clinics to determine the impact of these mobile web apps on informed decision making and uptake for the HPV vaccine.

Conclusion

This presentation will discuss the progress and initial results of these ongoing research efforts and the implications for reaching HPV vaccine uptake goals set by Healthy People 2020 in the United States. The presentation will focus on how web-
based interventions show promise for reaching HPV vaccine uptake goals. A mobile web app can make decision-making tools widely available on popular mobile platforms such as tablet computers and smartphones as well as personal computers.
Cervical cancer is one of the most common types of cancer in Croatia, especially among women between 20 and 49 years of age. Sixty five percent of all STIs occur in population under 25 years of age. HPV infection is the most common viral STI. In 2015, 111 cervical cancer related deaths were reported, meaning one woman every third day. These numbers alert for sexual education, HPV vaccination and cervical cancer screening.

Methods

The aim of this study is to present ten year pathway in implementing HPV vaccination in Croatian National Immunization Programme as voluntary and free of charge.

Results

The HPV vaccine has been registered in Croatia since 2007. The first recommendation for vaccination against HPV was published by Croatian Society for Gynecology and Obstetrics, for Cervical Cancer, Urogenital and STD and Dermatovenerology and Society for School and University Medicine. First HPV vaccinations were introduced in Zagreb, capitol city of Croatia.

In 2008 HPV vaccination highlighting the importance of school based health education on vaccine availability among pupils and their parents. Financed by local self-government, partially or totally, HPV vaccination becomes available in other parts of Croatia.

In 2009 Professional Associations of the Ministry of Health for the Prevention of Cervical Cancer and Other HPV Vulnerabilities was established and adopted guidelines for introducing the vaccine into the national vaccination schedule.

In 2010 Ministry of Health recommends vaccination against HPV to reduce the risk from HPV infection and the development of cervical cancer.
In 2015, in National Immunization Program was interpolated free of charge optional vaccination for 8th grade female students.

In 2016, Ministry of Health declares free of charge optional vaccination for male and female students in the 8th grade of primary school and 1st grade of high school, as well as catch up possibility.

From 2017, since HPV vaccination was registered in Croatia as well as on the impieducation of pupils and their parents through parental meetings in schools, information through polyvalent counsultation centers and additional school notifications (posters, info sheets) were provided. Average vaccine coverage varied between 5 and 15%.

Conclusion

All professional and scientific follow-ups show that awareness of personal responsibility and sufficient information are protective factors in young people’s sexual behavior. HPV vaccination proved to be highly efficient in cervical cancer prevention. However, low response to HPV vaccine in Croatia indicates necessity for revising existing public health programs.

References

Croatian Health Statistics Yearbook 2015, HZJZ, Childhood vaccine refusal and hesitancyintentions in Croatia: insights from a populationbased study, Anja Repalust, Sandra Šević, Stanko Rihtar & Aleksandar Štulhofer, 2015
HPV prevalence and associated risk factors in women with cervical pre-cancer and cancer in Switzerland at the beginning of the cantonal vaccination programmes: The CIN3+plus study

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Background / Objectives

The Swiss Federal Office of Public Health has recommended vaccination against human papillomavirus (HPV) to prevent cervical cancer since 2007. To monitor the future public health impact of vaccination, baseline population-based data are required. The objectives of this study were to determine the prevalence of HPV and examine associated risk factors in women with cervical intraepithelial neoplasia stage 3 or more severe lesions (CIN3+) in Switzerland.

Methods

We conducted a cross-sectional study with women diagnosed with CIN3+ in Switzerland. Ten pathology institutes from six cantons and three language regions participated. We conducted HPV typing on formaldehyde fixed-paraffin embedded specimens from 2014 and 2015. Women enrolled in 2015 were asked to complete a questionnaire. We described frequencies of HPV types. We also compared demographic characteristics and socioeconomic status (according to the Swiss neighbourhood index of socioeconomic position, Swiss-SEP) in the CIN3+plus group with the Swiss National Cohort (SNC) in 2014 and compared risk factors for HPV infection with the Swiss Health Survey (SHS) in 2012.

Results

We included 768 biopsies from 767 women aged 17-81 years with CIN3+ in 2014 and 2015. Of these, 745 (97.0%) were positive for any HPV type, 5 (0.7%) were negative and 18 (2.3%) were not evaluable. Overall, 475/768 (61.8%) biopsies contained HPV 16 and/or 18 and 687 (89.5%) contained an oncogenic HPV type covered by the nonavalent HPV vaccine (16, 18, 31, 33, 45, 52, 58). In 2015, 273 women completed a questionnaire. Compared with the SNC, fewer women with CIN3+ were born in Switzerland (49.0 vs. 63.4%; p<0.001) and more were single (48.9 vs. 28.1%; p<0.001), but mean Swiss-Sep index was similar (64.6±10.8 vs. 64.4±11.2).
65.2±10.9; p=0.135). Amongst women with CIN3+, higher proportions reported ≥2 sexual partners in the last 12 months (15.4% vs. 4.1%), smoking (38.5% vs. 22.0%) and hormonal contraception use in the last 12 months (35.5% vs. 22.4%) than women in the SHS.

Conclusion

This is the first study of HPV in women with CIN3+ covering all three language regions in Switzerland. Women with CIN3+ have levels of socioeconomic position that are similar to the Swiss general population but higher levels of some risk factors for HPV. Surveillance of HPV types in CIN3+ lesions is feasible and can be used to measure the future impact of HPV vaccination on clinical outcomes.

References

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https://www.bag.admin.ch/bag/de/home/aktuell/news/bag-bulletin.html,

Background / Objectives

Human papillomavirus (HPV) vaccination recently became available in Brazil for school-aged girls through the Brazil National Immunization Program. We evaluated acceptability of HPV vaccination for daughters among women at high risk for cervical cancer.

Methods

Between January and November of 2016, we conducted a cross-sectional survey of 500 women with abnormal cervical cytology to examine predictors of HPV vaccination acceptability for daughters. Participants had no history of HPV vaccination, and were referred for first colposcopy at Barretos Cancer Hospital, a large tertiary care hospital in the state of São Paulo, Brazil.

Results

Most women had heard of prophylactic HPV vaccination (71%) and cervical cancer (95%). However, only around one third (39%) were aware of a vaccine to prevent cervical cancer. Most respondents (86%) indicated they would definitely get HPV vaccination for their adolescent daughters. Respondents were more likely to intend to vaccinate if a doctor recommended it (85% vs. 58% for nurse; p<0.001). Most (82%) indicated they would have their daughter vaccinated if it were available at school, and a notable proportion of respondents preferred to have their daughters vaccinated at a public health clinic (35%) or gynecologist’s office (41%). Correlates of intent to vaccinate included being married or in a domestic partnership (OR 2.41; 95% CI, 1.36-4.19), having the belief that HPV vaccination did not increase sexual activity (OR 2.36; 95% CI, 1.31-4.18), and that obtaining vaccination was not difficult (OR 1.86; 95% CI, 1.05-3.27).

Conclusion

HPV vaccination of adolescent daughters was highly acceptable to a group of at-risk Brazilian women, including through school-located programs. National vaccination strategies in Brazil should emphasize HPV vaccination as a free, accessible, and
effective tool to prevent cervical cancer and encourage physicians to discuss HPV vaccination with their patients.
Background / Objectives

Women screened for cervical cancer in Sweden and Norway are currently treated under a one-size-fits-all programme, which has successfully reduced the incidence of cervical cancer but relies on guidelines for scheduling screening. Utilising the information in the screening registries, guidelines could be replaced with individual risk estimates based on screening histories and ancillary information about the attendants. Adjusting the screening density at an individual level this way allows for increased observation of high-risk individuals while freeing up resources that would otherwise be spent on low-risk individuals that do not benefit from a high screening frequency. In this study, we applied a method for stratifying women into risk groups using their screening histories, based on the Swedish screening programme, and validated these results with data from the Norwegian programme, adapting the method to make use of the detailed HPV tests and biopsy samples available there.

Methods

We previously developed a method for stratifying women into risk groups using their screening histories using the Swedish Quality Register for cervical cancer (NKCx). Each screening diagnosis was assigned a ‘risk score’ by an expert, and the history ‘risk score’ totals for each participant were computed by an algorithm that accounted for time and delay. The resulting method could identify some very high-risk individuals in the data (up to 15% of all Swedish cancer cases since 2001 were found in this group).

Results

Preliminary results show the same risk-identifying patterns in Norway as in Sweden, even though the screening programmes follow different clinical practices and guidelines. The ‘risk scores’ show exceptional probability of cancer in the upper ranges, and below normal risk in the lowest ranges. HPV status is often combined with low-risk diagnoses for prediction, but not high-risk, i.e. a result of normal is
treated differently if it’s HPV positive or negative, but the HPV status of a HSIL
diagnosis doesn’t matter for predictive purposes.

Conclusion

Preliminary results show strong similarities between Norway and Sweden in terms of
which screening patterns can be used to predict likelihood of cancer. This suggests
that the methodology is robust, and that somewhat different screening guidelines do
not affect the outcome of the algorithm as long as the data is accurate and complete.
Furthermore, the addition of HPV status divides diagnoses into HPV groups, enabling
more refined comparisons and predictions. As HPV tests become more widespread,
the algorithm is likely to improve in prediction accuracy and detail.

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| Xu X.Q.             | 00286  | FC 12  | 13     | 00289  | P13    | 06     |
| Xu H.               | 00169  | FC 06  | 03     | 00306  | FC 20  | 11     |
| Xu L.               | 00436  | FC 10  | 05     | 001     | 01      | 001     |
| Xu J.               | 00279  | P08    | 06     | 001     | 01      | 001     |
| Xuan S.             | 00543  | FC 21  | 03     | 001     | 01      | 001     |
| Xuan W.             | 00201  | SS 05  | 04     | 001     | 01      | 001     |
| Xue M.              | 00245  | FC 02  | 05     | 001     | 01      | 001     |
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Zhao Y. 00240 | FC 21-07, 00114 | P05-03
Zhao F.H. 00268 | FC 10-09, 00286 | FC 12-13, 00274 | FC 17-10, 00308 | FC 19-02, 00354 | FC 22-07, 00289 | P13-06
Zhao F. 00306 | FC 20-11
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