EUROGIN 2018 Abstracts

PART II - FREE COMMUNICATION SESSIONS

FC 01. Screening 1

00552

Molecular characterization of HPV16 sub-lineages: viral sequences, integration events, and human somatic mutation landscape

01. Viral and molecular biology

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Background / Objectives

Cervical cancer is the most frequent cause of cancer mortality for women living in poverty and is almost exclusively caused by the human papillomavirus (HPV). To better understand the molecular characteristics of HPV and the cervical tumor genome we have surveyed 665 cases of cervical cancer from Guatemala.

Results

Tumor DNA was sequenced by the capture of the exons of 245cancer-related genes, and variants called, and somatic mutation filtered. HPV 16 DNA was sequenced using a previously validated panel of overlapping primers, aligned to the viral genome and sub-lineage determined by phylogenetic analysis.

Conclusion

The average age is 52 and the number of children is 6.0, 5.6% report tobacco use; 56% have stage 2 or 3 cancer and 82% of tumors are squamous cell carcinoma. In total, 20% of subjects report a family history of any cancer and 6% for cervical cancer. Patients with a family history of cervical cancer have a younger age of onset

(47 years) and were pre-menopausal cancer (57% versus 42% in the entire sample). In total, 11/24 tumors have a mutation in the *PIK3CA* gene (46%) and 58% of tumors have at least one mutation in the PI3K pathway. Other known driver mutations include *TP53*, *RB1*, and *CASP8*. Mutations in chromatin remodeling genes (*PBRM1*, *EP300*, *SMARCA4*, *KMT2C*, *KMT2D*, *HIST1H3B*, *HIST1H4E*, *HIST1H1E*) are also elevated with 46% of tumors having at least one mutation. Tumors have an average of 18 mutations (median 14); however, 4 tumors have high (32-38 mutations) or very high (63-64) mutation load. All hypermutation subjects have *PIK3CA* mutations and are post-menopausal. Mutation signature analysis shows that the highest signature is for APOBEC-related mutations (46%) and HPV is known to activate APOBEC. Pre-menopausal patients have a lower mutation load (mean 13 mutations) and a lower fraction of APOBEC-related mutations (40%). Overall HPV16 accounts for 58% of HPV infections in Guatemala. We have sequenced and variant classified 96 HPV16+ cervical cancers and found that the D2 and D3 sub-lineages represented 26% and 29% of the samples, respectively. A total of 65% (62/96) of the samples had integrated HPV16 sequences as determined by HPV DNA capture and sequencing, and A1 and D2 sub-lineages showed a higher frequency of integration 78-79%) compared to D3 (44%). Subjects with HPV16 integration have a significantly younger age (P=0.009), and D2 was observed in younger patients, as compared to A1 (P=0.001).

References

Guatemalan cervical tumors have a similar profile of somatic mutations to those in the US, with a high frequency of *PIK3CA* mutations, and the very high-risk HPV16 D2, D3 sub-lineages.

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00556

3D Squamous Epithelial Tissue Culture System for Anti-HPV Drug Discovery and Validation

01. Viral and molecular biology

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Background / Objectives

Management of HPV lesions requires better therapeutic options than are presently available. We established a three-dimensional epithelial tissue culture system from primary human keratinocytes harboring HPV-18 replicons, fully recapitulating a robust infectious program (Wang et al. 2009). Systematic investigations of virus-host cell interactions in such 'raft' cultures grown at the liquid medium/air interface (Dollard et al. 1992; Wilson et al. 1992) have identified critical regulatory pathways on which HPV DNA amplification depends, revealing potential host targets for anti-viral therapies. Our strategy is to repurpose existing pharmacologic agents to inhibit viral DNA amplification, interrupt HPV transmission, or preferentially eradicate HPV-infected cells.

Results

Inhibitors are delivered to HPV-18 infected as well as to uninfected control PHK raft cultures either topically or through the tissue culture medium for up to two weeks before harvesting the tissues. In addition, durability of responses is evaluated after a post- exposure chase period. We then probe FFPE tissue sections for HPV DNA amplification, cellular DNA replication, papillomaviral proteins E6, E7 and L1, targeted host proteins, and tissue morphology, as well as for markers of DNA damage and apoptosis. Duplicate rafts are used for HPV DNA copy number evaluation to ascertain that there is a wide dynamic range between unimpeded viral DNA amplification and inhibited infections, typically 100-fold or more. Additional cultures can be used to evaluate specific mRNAs and micro-RNAs. Rafts can also be established from HPV-immortalized or -transformed epithelial cells or cervical cancer cell lines. Moreover, 3D cultures can be grown directly from patient lesions.

Conclusion

Based upon host cell metabolic and regulatory pathways essential for maintenance of the viral genome, replicative amplification and virion morphogenesis, we are systematically investigating inhibitors of mitogen-activated protein kinases, histone deacetylases, DNA Damage Responses including cell cycle checkpoints (Banerjee et al, 2011), replicative DNA amplification, and cytoplasmic vesicle function as well as inducers of stress responses. Using the 3D raft culture system, we have identified molecularly distinct inhibitor candidates as safe and effective. Several such agents are advancing to clinical trials to treat benign HPV lesions (Banerjee et al., 2018).

References

These proof-of-principle experiments demonstrate the potential for discovery of new drugs against epitheliotropic viruses. The authenticity of 3D experimental models of HPV infections and diseases should greatly reduce preclinical research time and expense.

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00630

Is HPV-negative cervical cancer a biologically different entity?

02. Epidemiology and natural history

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Background / Objectives

High risk human papillomavirus (hrHPV) infection is established as the major cause of invasive cervical cancer (ICC). Yet there seems to be a subset of cervical cancers where hrHPV is not readily detectable in the tumor tissue by standard PCR methods.

Methods

We recently described findings from an analysis of national registers and comprehensive HPV genotyping of all cervical cancer cases diagnosed in Sweden during the years 2002-2011.

Results

Of the 2845 included cases, hrHPV was detected in 2293 cases (80.6%) using general primer PCR with Luminex genotyping and real-time PCR targeting the E6/E7 regions of HPV16/18. Women with hrHPV-positive cervical tumors had a substantially better prognosis than women with hrHPV-negative tumors, independently of already established clinically relevant factors.

Conclusion

This raises the question whether L1 negative tumors are biologically different from L1 positive tumors. In this presentation, we will discuss different definitions of hrHPV

negativity, sensitivity of laboratory detection methods, and resulting implications for research and practice.

00057

Screening of cervical cancer in women aged 30 to 64 years screened with human papillomavirus tests (ESTAMPA* study). Experience in Paraguay.

09. HPV screening

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Background / Objectives

Paraguay has high incidence and mortality rates of cervical cancer of 34,2 y 15,7 x 100.000 women respectively.

The objective of this cross-sectional study was to assess the feasibility of implementing organized cervical cancer screening programs based on high risk human papilomavirus (HR-HPV) testing within the health system of Paraguay.

Results

The invitations to carry out the screening were made with house-to-house visits in communities of Itaugua and San Lorenzo, to women between 30 to 64 years old, period 2014-2017. All eligible women were invited to nearest health centers and institutions that had a room enabled to perform cervical samples collection to be screening by HPV test. Cervical samples were collected in PreservCyt medium and HR-HPV were deteted by Hybrid Capture 2. HR-HPV positive women were referred to colposcopy and, biopsies were taken in cases of abnormal colposcopic impressions. All the women with an anatomopathological diagnosis of cervical intraepithelial neoplasia (CIN2 +) were directed to treatment and all HR-HPV women with CIN 1 or less are being invited to a control visit at 18 months with the HPV test. The ESTAMPA study group received previous training to conduct home visits, sample collection, sample processing, colposcopic and anatomopathological evaluation. An internal and external quality control system were established.

Conclusion

A total of 5321 women were enrolled in the study, corresponding an average of 59% of elegible women invited to carry out the screening with a minimum participation of 44% and a maximum of 78% per community included. The prevalence of HR-HPV was 14%. Ninety percent of women attended the colposcopy visit and 1.3% were CIN2 +. Seventy-nine percent were treated by the ESTAMPA study group gynaecologists and the rest of CIN2+ women reported receiving treatment at other health centers.

References

It was possible to carry out an organized screening model achieving, an average of 59% participation in screening, 90% compliance with colposcopy among HR-HPV positive women and identifying a high prevalence of 1.3% of CIN2+. The ESTAMPA study group was able to treat 79% of cases. These results suggest the importance of strengthening screening and follow-up of women at risk of developing cervical lesions through organized programs based on sensitive molecular tests.

References

* ESTAMPA study: a multicenter study of screening and triage of cervical cancer with human papillomavirus tests.

00080

HPV- DNA primary screening in Israel decreased colposcopy referrals – The experience of Maccabi Health Medical Organization

09. HPV screening

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Background / Objectives

The primary cytological screening for detecting cervical cancer and precancerous lesions (PAP-smear) has been proven in reducing morbidity and mortality of cervical cancer when performed once every 3 years. The main disadvantage of cytology is its low sensitivity. In order to improve the sensitivity of the survey to detect malignant and pre-malignant lesions it has been proposed to use HPV-DNA primary screening instead. Maccabi HMO (220000 affiliates, 25% of the Israeli population) started to use HPV-DNA primary screening based on HPV DNA only test (cobas test , roche) at 1/3/18, with triage of cytology and genotyping for the referral to colposcopy.

Objective: To compare the rate of referral to colposcopy based on two primary screening methods (HPV vs PAP tests) in the Israeli population.

Results

Data was collected for each screening-group based on a centralized computerized system. The sreening was done for women aged 25-65 in both groups. The time periods were 1.1.2017 to 31.12.2017 for the cytology screening, and 1.3.2018 to 21.5.2018 for the HPV-DNA screening. In the primary-cytology era, women with abnormal cytology (>LGSIL) or accasionaly with ASCUS, were referred for colposcopy. During the primary HPV-DNA screening, women that are found positive for HPV 16/18 or positive for other High-Risk HPV with abnormal cytology were referred for colposcopy. We are comparing the rate of referral for colposcopy during the two methods of primary screening

Conclusion

21,033 women with primary-HPV-screening tests during the study period, were compared to 112000 women screened by primary-cytology. In the primary HPV-DNA screening group 726 women (3.45%) were referred for colposcopy, of which 2% were positive for HPV 16-18 and 1.45% positive for other HR-HPV with abnormal cytology. In the primary cytology screening era 7392 women (6.6%) were referred for colposcopy of which 4.1% had abnormal cytology > LGSIL and the rest (2.5%) from the ASCUS group. The differences were found to be statisticalyl significant by chi-square analysis (OR=0.51, 95% Confidence Interval, 0.46-0.56, p-value < .00001) favoring primary-HPV screening

References

Primary HPV-DNA screening in Israel decreases significantly the rate of referral for colposcopy, when compared to primary-cytology screening.

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00101

IMPLEMENTATION OF PRIMARY HPV mRNA SCREENING FOR CERVICAL CANCER: FIRST YEAR EXPERIENCES

09. HPV screening

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Background / Objectives

Primary HPV screening for cervical cancer by HPV mRNA testing (APTIMA) was implemented in January 2017, for women ≥30 through 70 years in the region of Skåne, Sweden. HPV positive samples underwent cytology triage, and women with abnormal cytology for atypical squamous cells of undetermined significance + (ASCUS+) were referred to colposcopy. The aim was to perform an audit of the primary HPV screening program year 2017, and to compare its cytology results to that of corresponding women aged ≥30 through 65 years screened with cytology during 2016.

Results

The present audit was register-based. The Aptima HPV assay (Hologic) was performed according to the manufacturer's instructions using the Panther platform (Hologic). Primary HPV screening starts at >30 years. Women not attending the final invitation at 65 years receive re-invitations up 70 years. During 2017 62,971 women were analysed for presence of HPV within the primary HPV screening. In order to ensure that the primary HPV screening program also detects the few cases of cellular changes that may occur without an active HPV infection 5,027 women aged 40-42 years were co-tested. We compared proportions of abnormal cytology between cytology screening and primary HPV screening of women aged 30-65 years. Data from cytology screening (N=45,754) were collected from 2016, and from primary HPV screening during 2017 (N=49,774). The cytology screened and the primary HPV screened women had the similar age distribution (Table 1).

Table 1.Proportion of women stratified by age.

AGE	2016 Cytology#	2016 Cytology Proportion %	2017 HPV#	2017 HPV Proportion%
30-39	16664	36.4	17287	34.7
40-49	16132	35.3	16075	32.3
50-65	12968	28.3	16412	33.0
Total#	45764	100	49774	100

Conclusion

HPV was detected among 7.0% (4,422/62,976) of the women 2017. Among a control group of the screening population with co-testing (cytology and HPV) aged 40-42 years (N=5,027), HPV was detected in 100% (28/28) of high-grade squamous intraepithelial lesion (HSIL) and among ASCUS where HSIL could not be excluded (ASC-H) (9/9), and 80% (4/5) of atypical glandular cells (AGC). Thus, within this group the sensitivity of the HPV mRNA test to detect severe dysplasia was 98% (95% CI; 87% to >99.9%).

The proportion ASCUS+ was 3.38% and 3.70% with cytology and primary HPV screening, respectively (P=0.0080). Only the proportion of ASC-H cytology changed by the use of primary HPV screening, from 0.13% to 0.23% (p<0.001).

References

Primary HPV mRNA screening for cervical cancer screening marginally increased (0.32%) the proportion of women with ASCUS+ cytology.

Cytology decreased by 83% within our primary HPV screening of women aged 30 through 70 years, due to the relatively low amount of cytology triaged samples and the co-testing cytology performed.

00113

RISK OF CIN2+ AFTER A NEGATIVE 1-YR RECALL HPV TEST IN HPV-POSITIVE WOMEN WITH NORMAL CYTOLOGY ATTENDING HPV CERVICAL SCREENING

09. HPV screening

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Background / Objectives

In Italy, women screened by HPV testing undergo cytology triage in case of positivity; those with normal cytology repeat HPV testing after 1 year, and return to routine screening in case of negativity. We evaluated their subsequent CIN2+ risk in order to define a safe screening interval.

Results

We analyzed the data from four Italian pilot projects (Alta padovana, Monselice, Padova and Vallecamonica) that nested HPV screening implementation within organized cervical cancer screening programs, enrolling 25-64 yrs-old women presenting for a new screening round. Search for high-risk HPV (hrHPV) DNA was performed by Hybrid Capture 2 (HC2, Qiagen). According to the protocol, both HPV-negative women and HPV-positives testing negative at 1-yr repeat returned to screening after three years. We compared HPV test positivity, CIN2+ and CIN3+ detection at 3-year re-screening in the 688 women with such transient infections to those in the 53,405 women HPV-negative at the previous screen.

Conclusion

At the second screening round, among women with previous transient infection, 105 tested HPV-positive and 6 CIN2 and 2 CIN3, but no cervical cancer, were detected. Compared with previously HPV-negative women, HPV infection among women transiently HPV-positive was four times more frequent (15.3‰ vs 3.9%, respectively. Relative Risk 3.93; 95%CI 3.15-4.85). The detection of CIN2 was fourteen times

more frequent (8.7‰ vs 0.6‰, respectively. RR 13.7; 95%CI 4.7-33.1) and the detection of CIN3 eight times more frequent (2.9‰ vs 0.4‰, respectively. RR 7.8; 95%CI 0.9-32.0).

References

Women with hrHPV infection without cytological abnormalities and undetectable HPV at short-term repeat have higher rates of HPV infection and of detection of CIN2 and CIN3 lesions at the subsequent screening round. According to European and Italian guidelines, these women return to regular screening, and some concern has been raised on the safety of a five-year interval. At a 3-year re-screening, we observed higher rates mainly of CIN2 lesions. The detection of CIN3 is similar to that observed in routine European cytology-based screening programs (2.7‰), showing that this level of risk is considered as acceptable. The evaluation of results from other pilot projects is planned.

00230

SAFETY AND EFFICACY OF PROPHYLACTIC HPV VACCINES. A COCHRANE REVIEW OF RANDOMISED TRIALS

09. HPV screening

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Background / Objectives

Recently, the evidence on efficacy and safety of prophylactic HPV vaccines derived from randomised controlled trials (RCTs) was published in the Cochrane database of Systematic reviews. A summary of this Cochrane review is presented below.

Results

Only RCTs involving mono-, bi- and quadri-valent HPV vaccines were included. Trials evaluating the nona-valent vaccine were excluded since women in the control group received the quadri-valent vaccine. Main outcomes were: histologically confirmed cervical precancer lesions distinguishing those associated with vaccine HPV types and any cervical precancer. Exposure groups were: young women (15-26 years) or mid-adult women (24-45 years) being initially negative for high-risk HPV (hrHPV) or negative for HPV types included in the vaccine and women unselected by HPV status.

Conclusion

All evaluated vaccines offered excellent protection against cervical intra-epithelial neoplasia of grade 2 or 3 (CIN2/CIN3) and adenocarcinoma-in-situ (AIS) associated with HPV16/18 infection in young women who were not initially infected with hrHPV or HPV16/18. Vaccine efficacy decreased when women regardless of HPV DNA status at enrolment were included. Vaccine protected also in young women but at a lesser degree against any cervical precancer. Vaccine efficacy was lower in mid-adult women. Trials were not empowered to address protection against cervical cancer. Occurrence of severe adverse events or adverse pregnancy outcomes was not significantly higher in recipients of HPV vaccines than in women included in the control arms.

References

To complete evidence from randomised trials, careful population-wide surveillance of HPV vaccine effectiveness (targeting also incidence of HPV-related cancers) and safety (including also rare conditions such as neurologic and auto-immune syndromes) should be set up by linking vaccination, cervical cancer screening and morbidity registries.

References

KEYWORDS: Cervical cancer, HPV vaccines, safety, randomised clinical trials, systematic review, meta-analysis.

00317

Accuracy of high-risk HPV testing and HPV16/18 genotyping to triage women with LSIL: a pooled analysis of VALGENT studies

09. HPV screening

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Background / Objectives

Genotyping for the most carcinogenic HPV types (HPV16 and HPV18) could identify those women at highest risk requiring colposcopy or more intensive follow-up in women with low-grade squamous lesions (LSIL).

Results

The VALGENT framework is designed to assess the analytical and clinical performance of HPV tests that offer limited to extended genotyping capability. VALGENT is iterative using panels collated in different countries. A pooled analysis was performed, using data from three completed VALGENT panels, to assess the diagnostic accuracy of genotyping for HPV16/18 and for hrHPV (13 or 14 types) to detect prevalent CIN2+ in women with LSIL. Data pooling was performed using a bivariate normal model designed for meta-analysis of diagnostic test accuracy, taking the intrinsic negative correlation between sensitivity and specificity into account.

Conclusion

Twenty HPV tests were evaluated within three VALGENT panels. The pooled sensitivity and specificity of hrHPV in aggregate to detect CIN2+ was 98.1% (95%CI: 95.5 -99.2%) and 23.8% (95%CI: 20.6-27.3%) in women with LSIL, respectively. HPV16/18 genotyping had a sensitivity and specificity for CIN2+ of 56.2% (CI: 51.0-61.3%) and 77.1% (CI: 73.0-80.8%), respectively. HPV16/18 genotyping was substantially more specific (ratio: 2.65, 95%CI: 2.13-3.28) but also less sensitive than testing for hrHPV (ratio: 0.62, 95%CI:0.57-0.68). No significant inter-panel

differences were observed either for the pooled analysis of hrHPV test accuracy or theHPV16/18 genotyping. The average risk of underlying CIN2+ was 39.7% in HPV16/18-positive women with LSIL, 13.1% in women who were HPV16/18-negative but positive for other hrHPV types and 2.1% for hrHPV-negative women.

References

Triage of women with LSIL with partial genotyping identifying HPV16/18 increases the PPV compared to hrHPV but at the expense of lower NPV. Women testing positive for HPV16/18 need further diagnostic and/or therapeutic work-up. Women testing HPV16/18-negative but positive for other types may also be referred to colposcopy whereas hrHPV-negative LSIL patients also should be kept under surveillance. Further development and optimization of triage markers are needed to manage women with LSIL.

00320

mRNA HPV E6/E7 SCREENING: A 3-YEAR LONGITUDINAL COTEST STUDY IN MADRID. PRELIMINARY RESULTS.

09. HPV screening

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Background / Objectives

There are few studies measuring the risk of a high-grade (CIN2+) lesión after a negative Aptima®high-risk mRNA HPV test (AHPV) (1-3). In the first published pilot study in Madrid, a baseline screening cotest with cytology and AHPV on 5.053 women aged 25-65, yielded a 9% AHPV prevalence and a 26,3% rate of CIN2+ lesions in AHPV-positive biopsied women (4).

The objective of the present study is to analyze the negative predictive value (NPV) of the mRNA HPV test with a longitudinal analysis at 3-years of the previously screened population.

Results

So far, 520 women with a baseline AHPV-negative result have been actively recruited by phone call for a new cotest analysis 3 years later. Cervicovaginal samples were obtained from a health care professional and placed into ThinPrep® PreservCyt solution. Simultaneous cytology with a ThinPrep® 5000 processor and AHPV analysis with the automated Panther® platform was performed. AHPV-positive women or those with LSIL+ cytology results were referred to colposcopy and biopsy or endocervical curettage.

Conclusion

The prevalence of AHPV in this longitudinal phase was 3,8% (20/520) and the age was significantly lower in AHPV-positive than in HPV-negative women (41,4 vs 46,15 years, p=0,017). There was a histological analysis in 65% of AHPV-positive women (13/20), yielding 4 CIN1 and 1 CIN3 lesions. From these, only the woman with a

CIN3 had abnormal cytology (HSIL). From the 500 AHPV-negative women, there were 3 cases with cytological ASCUS and 1 with an LSIL diagnosis (0,8%).

References

The prevalence of AHPV decreased from 9% in the baseline study to 3,8% three years after a negative AHPV test. Infected women were significatly younger than those AHPV-negative ones, suggesting new infections. The risk of harboring a CIN2+ lesion in the AHPV-positive group of the longitudinal study was 7,7% as compared to 26,3% of the baseline. Therefore, preliminary results of this study demonstrate a low risk of a high-grade cervical lesion 3 years after a negative AHPV test.

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00363

CERVIVA HPV Primary Screening Pilot Study: evaluation of triage strategies for HPV-positive women

09. HPV screening

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Background / Objectives

Triage of HPV positive women is one of the key challenges facing HPV primary screening. Specific second round triage tests to avoid large numbers of unnecessary referrals to colposcopy are required. This study investigates a panel of triage options including HPV16/18 genotyping, cytology and dual staining for p16/Ki-67 in women who test positive for HPV in primary screening.

Results

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 were tested for HPV DNA (cobas 4800 HPV test) and mRNA (Aptima HPV assay). All HPV-positive women were further assessed with HPV16/18 genotyping, cytology and p16/Ki-67 dual staining. The performance of different triage strategies was examined both cross-sectionally and will be longitudinally assessed over two screening rounds for detection of CIN2+.

Conclusion

12,608 eligible woman have been recruited into the study. The median age of the population is 38 years. HPV DNA testing, performed on 10,528 samples, shows a 15.7% positivity rate. HPV mRNA, performed on 12,601 samples, gave a 12.8% positive rate. Overall, 31.2% (514/1650) of HPV DNA positive women were positive for HPV16/18, 33.2% (548/1650) had an abnormality on cytology and 32.8% (274/836) tested positive for p16/Ki-67. p16/Ki-67 demonstrated the highest sensitivity and specificity for detection of CIN2+ (0.91, 0.78 respectively), when combined with HPV16/18 genotyping sensitivity was similar but specificity was significantly reduced (0.93, 0.67 respectively).

References

Here we present the preliminary cross-sectional data in relation in to each of the putative triage tests. p16/Ki-67 appears to be a sensitive and specific triage test for women who test positive for HPV in primary screening.

00396

ABSOLUTE AND RELATIVE RISK OF CIN2/3+ IN WOMEN ASCUS HPV16/18+ VERSUS ASCUS 12OTHER HRHPV+: BASELINE RESULTS OF THE COMPACT STUDY−

09. HPV screening

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Background / Objectives

While cytology-based screening programs have significantly reduced mortality and morbidity from cervical cancer, the global consensus is that primary human papillomavirus (HPV) testing for cervical screening increases detection of high-grade cervical intraepithelial neoplasia (CIN) and invasive cancer. However, the optimal triage strategy for HPV positive women to avoid over-referral to colposcopy may be setting specific. As Japan requires data generated domestically to modify screening guidelines, we conducted a 3-year prospective study, COMparison of HPV genotyping And Cytology Triage (COMPACT), to evaluate the potential role of HPV16/18 partial genotyping and cytology for primary HPV screening. This study compares absolute and relative risk of ≥CIN2/3 at baseline in women ASCUS HPV16/18+ compared to those ASCUS 12other hrHPV+.

Results

Participants were 14,642 women aged 20-69yrs attending for screening at Hokkaido Cancer Society in 3 cities in Hokkaido, Japan, between April 2013 and March 2014. Women with ASCUS or worse cytology, regardless of HPV status, and NILM HPV16/18+ went straight to colposcopy. Women NILM 12other hrHPV+ underwent repeat cytology after 6m and those with ≥ASCUS went to colposcopy. The present analysis focuses on 14,500 women aged 25-69yrs.

Conclusion

Totally, 150 (1.03%) women had ASCUS cytology. Mean age was 44.4 yrs. Overall, 86 cases were hrHPV- and 64 hrHPV+. In the latter, 19 (29.7%) women were HPV16/18+ and 45 (70.3%) had a 12other hrHPV infection. Absolute risk of ≥CIN2 and ≥CIN3 in women HPV16/18+ was 71.42% (95% CI: 45.35%-88.28%) and 50.0% (95% CI: 26.8%-78.2%), respectively. In women with a 12other hrHPV type it was 21.43% (95% CI: 10.21%-39.54%) and 7.14% (95% CI: 1.98-22.64%), respectively. Absolute risk of CIN3 with HPV negative ASCUS cytology was 2.17% (95% CI:0.38%-11.33%). There were no invasive cervical cancers. Relative risk (RR) of ≥CIN2 and ≥CIN3 in women with an HPV16/18 infection compared to a 12other was 3.33 (95% CI: 1.52-7.29: p=0.003) and 7.0 (95% CI: 1.67-29.39, p=0.008), respectively.

References

The prevalence of ASC-US among women in the COMPACT study was lower than ATHENA, but similar to other Japanese studies. Similar to other global studies, absolute risk of high grade lesions was significantly higher with an HPV16/18 infection compared to a 12 other or no HPV infection. Absolute risk of ≥CIN2 in women ASCUS 12 other+ was similar to that of women HPV16/18 positive with NILM cytology in the COMPACT study, 21.43% versus 19.54%. Furthermore, while women NILM HPV16/18 had a 1.5 fold higher risk of CIN3 compared to women ASCUS 12other, it was not statistically significant. (RR, 1.54, 95% CI: 0.35-6.66, p=0.57). Prospective data from the COMPACT trial is needed to decide the most appropriated triage strategy for HPV+ women.

00461

A RISK-BASED APPROACH: CO-TESTING 34 612 WOMEN WITH CYTOLOGY AND 3-TYPE HPV MRNA TEST

09. HPV screening

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Background / Objectives

HPV DNA testing is more sensitive, but less specific than cytology. HPV DNA test cannot be used in young women due to a high positivity rate. A 3-type HPV mRNA test is more specific. We wanted to investigate if co-testing could provide better protection among all age groups; reducing false negatives and enhance risk stratification by detecting HPV mRNA genotypes 16, 18 and 45, the three most prevalent HPV types in cervical cancer.

Results

From April 2016 to December 2017, the Department of Pathology, University Hospital of North Norway, 34 612 women were co-tested with cytology and a HPV E6/E7 mRNA test detecting 16, 18 and 45 (PreTect SEE). Women were followed up until June 2018. Histologically confirmed CIN2+ were used as study endpoint.

Conclusion

At baseline, 993 (2.9%) had positive HPV mRNA, 5 297 (15.3%) had abnormal cytology (ASC-US+) and 760 (2.2%) had confirmed CIN2+ during follow-up. The PPV for CIN2+ of cytology, HPV mRNA-test and double positive (Cyt+/HPV+) were 13.3% (702/5297), 37.9% (376/993) and 49.0% (333/679). HPV mRNA positive rates in the age groups 14-24y, 25-33y, 34-49y and 50-69y were 10.1%, 6.0%, 1.8% and 0.8% and abnormal cytology rates were 38.2%, 24.0%, 15.0% and 6.8%. The detection rates of CIN2+ were 5.2%, 5.2%, 1.6% and 0.5%. The PPV for CIN2+ of cytology were 12.7%, 20.3%, 9.8%, 5.9%; HPV mRNA test 26.5%, 45.7%, 37.6%, 26.0% and double positive (Cyt+/HPV+) 33.1%, 56.9%, 49.0% and 42.5%.

References

The 3-type HPV mRNA test is more specific than cytology and holds high PPV for CIN2+ regardless of age. The low positivity rate enables HPV mRNA testing of women not eligible for HPV DNA testing. Co-testing versus cytology alone gives a significant increase in PPV for CIN2+ (49.0 % vs 13.3 %) hereby improving safety and providing important assistance for clinicians in determining patients elevated risk and need for follow-up. A risk-based approach may reduce over referrals and overtreatment.

00464

PRIMARY CERVICAL CANCER SCREENING WITH A 5-TYPE HPV E6/E7 MRNA TEST: RESULTS OF 10 YEARS FOLLOW-UP

09. HPV screening

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Background / Objectives

To assess the performance of a 5-type HPV mRNA test in primary screening for women 25-69 years. We estimated the cumulative risk of CIN3+ (%) at 42, 78 and 120 months.

Results

In 2004, 19 153 women were recruited for participation in a primary screening study with a 5-type HPV mRNA test. The HPV test targeted E6/E7 mRNA from HPV 16, 18, 31, 33 and 45 (PreTect HPV-Proofer). After excluding women with a history of abnormal cytology/CIN2+; 9 582 women were eligible for study participation. The Norwegian Cancer Registry completed follow-up of CIN2+ through December 31st 2015.

Conclusion

At study start, 27.2% of the women were 25-33 years, respective 72.8% 34-69 years old. In total, 3.2% were HPV mRNA positive, 1.5%, 0.5%, 1.2% for HPV 16, 18, and 31/33/45, respectively. The cumulative risk of CIN3+ at 42, 78 and 120 months (1, 2 and 3 screening intervals) were 11.3% (95% CI: 7.6-14.9), 16.9% (95% CI:12.5-21.2), 25.3% (95% CI: 19.0-31.5) for HPV-positive women, and 0.32% (95% CI: 0.31-0.33), 0.66% (95% CI: 0.64-0.68), and 0.99% (95% CI: 0.96-1.02) for HPV-negative women, respectively.

References

HPV mRNA positive women have a significant elevated risk of CIN3+ and can be referred directly to colposcopy and biopsy. Test negative women have low risk of CIN3+ and may return to screening. Low HPV mRNA positivity rate implies low

referral rates and reduced risk of over-treatment. A trade-off between sensitivity and specificity must be considered when decisions on HPV tests in primary screening are taken.

00582

3-TYPE HPV MRNA TEST IN DETECTION OF CIN2+ IN YOUNG WOMEN WITH NORMAL CYTOLOGY

09. HPV screening

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Background / Objectives

Objectives. Despite a well-established cervical cancer (CC) screening program in Norway, the incidence of CC in young women is increasing, peaking at 35 years. Twenty-five percent of all women diagnosed with CC had normal cytology within 3 years of cancer diagnosis, addressing the need of improvement of screening program to further reduce cancer incidences missed by cytology. We wanted to investigate the detection rate of CIN2+ in young women with normal cytology by using a 3-type HPV mRNA test.

Results

Methods. In 2014-2017, 2,382 women <40 years with normal cytology at Nordlandssykehuset-Bodo,Norway, were HPV-tested using a 3-type HPV E6/E7 mRNA test (PreTect SEE; direct genotyping 16, 18 and 45). Index cytology from women with a positive HPV mRNA test were rescreened. Women with revised cytological diagnoses were followed-up according national guidelines until August 2018. We used histologically confirmed CIN2+ as study endpoint.

Conclusion

Results. Overall, 2.1% (50/2,382) had positive HPV mRNA test. The cytology was revised in 52.0% (26/50); 11 ASC-US, 6 LSIL, 1 AGUS, 6 ASC-H and 2 HSIL. During follow-up, biopsy was taken from 30 women. CIN2+ was detected in 56.6 % (17/30) from women that testedHPV mRNA positive (8 CIN2 and 9 CIN3), giving a total prevalence of CIN2+ of 0.7% (17/2,382) in presumed cytology normal women.

References

Conclusions. By testing women <40 years with normal cytology with a specific 3-type HPV mRNA test, an increase in screening program sensitivity can be achieved without an excessive workload. The volume of rescreened smears is low (2.1%). The PPV for CIN2+ is high (56.6%). When more women with CIN2+ are detected and treated in the first screening round, less women will develop cervical cancer before next screening round.

00075

RNA SEQUENCING OF HUMAN PAPILLOMAVIRUS NEGATIVE INVASIVE CERVICAL CANCERS

11. Genotyping

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Background / Objectives

Although cervical cancer is known to be caused by human papillomavirus (HPV), some tumors appear to be HPV-negative by primer-based detection systems.

In a previous study, we identified and requested FFPE blocks from all cervical cancers in Sweden during 2002 to 2011 (n=4254). Out of the 2850 cancer cases with adequate HPV typing results, there were 394/2850 (13,8%) cases being “apparently HPV-negative” after being tested for HPV DNA with both PCR with MGP primers targeting L1 gene and real-time PCR with primers targeting the E7 gene. We wish to perform unbiased testing (not based on PCR or other methods requiring prior knowledge of sequences) to see which actively transcribed viruses could be found in “apparently HPV-negative” cancer cases.

Results

As a pilot study, we included six cervical specimens “apparently negative” for HPV. Cervical specimens were RNA extracted with a xylene-free method, rRNA depleted, reverse transcribed and ligated to individual adapters using the TruSeq Stranded Total RNA kit (Illumina, US). Libraries were validated, normalized to 2 nM and pooled before sequencing. Sequencing was performed in the NextSeq500 system (Illumina, US) at 151 paired-end cycles. 150 bp long quality reads were screened against the human reference genome hg19 and human reads were filtered from the data set. Fastq files for each sample, were aligned to all HPV types reference clones sequences published in the website of the International Human Papillomavirus Center (hpvcenter.se, accessed 2018-05-28).

Conclusion

3/6 samples were positive for HPV RNA, with HPV 213 (Gamma-13), HPV 197 (Gamma-24) and HPV type 16 (Alpha-9) being found in one specimen each. While HPV 197 had 3524 reads covering all HPV genes (E6, E7, E4, E2, E1, L2 and L1), the HPV 213 and HPV 16-positive specimens showed reads only mapping to their respective E1 genes.

References

In Illumina total RNA sequencing data with a median of 30 million reads per sample, HPV transcription was detected in 3/6 apparently “HPV-negative” cervical cancer specimens (negative in PCRs directed to the L1 and E7 regions). The HPV197 and HPV213 may have escaped detection due to mismatch to primers/probes in the conventional PCR-based HPV detection systems.

00062

Online platform for monitoring of cervical screening programmes in the Nordic countries

13. Screening methods

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Background / Objectives

Quality assurance and improvement of cancer screening programmes require up-to-date monitoring systems and evidence-based indicators that reflect benefits, costs and harms. [1] The NordScreen project has developed a publicly available web-based interactive application to access standardized performance and outcome indicators of cancer screening, based on up-to-date Nordic cancer screening register data. [2] Fact sheets summarising the cancer screening policies and programmes in place in all the Nordic countries and Estonia are also available on the NordScreen website.

Results

The screening data originate from population-based screening registries. The test data are available on individual level linked to personal ID number. Through a network of Nordic and Baltic screening managers, population-based individual screening data from each country were converted to standard format in situ and aggregated by an R program script for use by the NordScreen online platform. Comparability between countries is enhanced by the uniform data structure and standardized calculations. The online platform is currently based on PowerBI software by Microsoft.

Conclusion

The NordScreen collaboration has so far collated standardized indicators for test coverage, average number of tests per interval and distribution of women according to number of tests per interval. These indicators are based on 32.8 million screening

tests from 4 Nordic countries and Estonia. Interactive comparison of test coverage between countries is currently possible on-line (nordscreen.org). In 2015, the test coverage within a time interval of of 5.5 years in the age group 30–64 year-olds was between 78–84% in Iceland, Norway and Sweden whereas 70% in Finland. The application allows users to define indicator specifications interactively.

References

NordScreen is a pilot model for cross-country comparison of cancer screening. The comparison of coverage rates to national figures show that the methods produce quite similar results. The aim is to stimulate collaborative research and quality improvement in screening through freely available, interactive, and regularly updated quality indicators. The project currently includes data on cervical cancer screening and screening programmes for breast cancer and colorectal cancer will be included in coming phases.

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00436

p16/Ki-67 AND HPV AS TRIAGE TESTS IN ROUTINE SCREENING: CORRELATION WITH HISTOLOGY

13. Screening methods

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Background / Objectives

Annual conventional cytology (CC) is still the standard in the German cervical cancer prevention program. The sensitivity of a single CC for the detection of cervical intraepithelial neoplasia is low. High-risk HPV (HR) testing and p16/Ki-67 (CINtec® Plus, Roche Ventana, Mannheim, Germany) are valuable for the triage of borderline and low-grade cytological abnormalities. Both tests used in routine improved the sensitivity and specificity for CIN 2+ lesions. We analysed the correlation between the p16/Ki-67 and HPV status of patients before biopsy/therapy with histologically confirmed CIN 2 and 3 and invasive cervical carcinoma.

Results

All cases of a German routine lab in which histology had been performed from 2012 till 08.2018 and results of, for HR HPV DNA tests (Hybrid Capture2 ® Qiagene. Hilden, Germany till 2013 after that cobas ®, Roche Diagnostics, Mannheim, Germany) and p16/Ki-67 tests (both carried out maximally 3 months earlier) were available are reported. Histology followed colposcopically directed biopsy, conization. All HPV and p16/Ki-67 tests were made out of cervical smears taken in proprietory tubes or in Thinprep vials (PreservCyt®, Hologic, Wiesbaden, Germany) according to the manufacturers instructions. While cytology, HPV- and p16/Ki-67 tests were made at Cytomol, histology was performed in numerous pathology institutes.

Conclusion

In 5354 of 6697 (80%) CIN 2+ cases an HPV result and in 3737 (56%) cases a p16/Ki-67 result was available. For all years together in CIN 2 1246 of 1295 (96,2%), in CIN 3 3794 of 3918 (96,8%) and in cervical cancer 133 of 141 (94,3%) cases were HR HPV positive. In CIN 2 832 of 864 (96,2%), in CIN 3 2775 of 2793 (99,35%) and in cervical cancer 79 of 80 (98,75%) cases were p16/Ki-67 positive. Altogether 181 (3,3%) histologically confirmed CIN 2+ were HPV test negative, and in 51 (1,3%)

were p16/Ki-67 negative. Only 7 of 57 CIN 2+ HPV high risk test negative cases with parallel p16/Ki-67 testing were negative for both tests.

References

The large majority of histologically confirmed CIN 2 and 3 and cervical cancers were positive for HR HPV and the biomarker p16/Ki-67 when tested in cervical smears < 3 months before biopsy/therapy. These results from routine testing point to a high sensitivity of HR HPV testing for CIN 2+ and slightly higher sensitivity of p16/Ki-67 (in CIN 3 and ICC cases). Triaging with HPV and p16/Ki-67 in routine increased sensitivity and specificity for CIN 2+ and may avoid the overtreatment.

00394

MUCH LOWER RATE OF LIMITED AND INSUFFICIENT SMEARS WITH LBC (THINPREPTM) THAN WITH CONVENTIONAL CYTOLOGY - EXPERIENCE IN ROUTINE

14. Liquid based cytology

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Background / Objectives

While the sensitivity of liquid based cytology (LBC) for high grade cervical disease is still under discussion the better technical quality of its smears is much less questioned. Most of these analyses have been performed under study conditions. We therefore investigated that aspect in a large routine lab.

Results

Cytomol is one of the major cytology labs in Europe with an experience in LBC (ThinprepTM) since over 15 years. Since over ten years all LBC and conventional specimens are pre-analyzed by computer-assistance. 12 of our cytotechnicians (CTAs) were asked to qualify all routine slides they examined within two weeks with special consideration to the technical quality of the smears. Because in Germany LBC is still reserved to privately insured and self-paying patients its volume is only about 10% of all cytology cases. This is reflected in the following data.

Conclusion

The age range of the CTAs examining conventional slides was 24 to 58 years, their professional experience varied from 3 to 35 years. 11.887 slides were analyzed, the individual CTA saw between 61 and 3394 slides. Alltogether 83.63 % of them were classified as well evaluable, 14.53 % as of limited and 1.84 % as of insufficient evaluability. The reasons for that judgement were (in decreasing frequency) overlay by leucocytes and blood and/or mucus, fixation failure, mechanically alterated cells and lacking endocervical cells. The median of the percentage of the well evaluable cases was 84.11%, its range 63.16 % to 95.08 %. For the cases of limited evaluablity

the median was 15.78 %, its range 4.92 % to 29.32 % and for the smears qualified as insufficient the median was 0.51 %, its range 0 % to 7.52 %.

1.193 LBC specimens were examined by 7 CTAs whose age ranged from 40 to 58 years and whose professional experience varied from 11 to 35 years. The individual CTA saw between 60 and 382 slides. 93.30 % of them were classified as well evaluable, 5.53 % as of limited and 1.17 % as of insufficient evaluability. The reasons for that judgement were (in decreasing frequency) use of gel, cytolytic cells and low cell number. The median of the percentage of the well evaluable cases was 94.41 %, its range 87.96 % to 100 %. For the cases of limited evaluability the median was 4.20 %, its range 0 % to 11.11 % and for the smears qualified as insufficient the median was 0.93 %, its range 0 % to 2.99 %.

Together the percentage of smears with limited or insufficient technical quality was 16.38 % for conventional slides and 6.76 % for LBC.

References

In routine use LBC had a 2.42 times lower rate of smears with limited or insufficient evaluability than conventional cytology.

00202

Nine years of the SCOTTISH HPV ARCHIVE - A resource support for basic and applied HPV research

19. New technologies

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Background / Objectives

Biobanking is essential to support HPV-associated basic and clinical research. A recent survey of key opinion leaders confirmed this as a top 10 priority for HPV based research and development. Some of the key considerations for biobanking are: to ensure samples are stored and disseminated with due process of governance; ensuring samples are of the nature and quality to support contemporary, priority research; and sustainability models.

Results

The Scottish HPV Archive received government core-funding for the first five years and then has been sustained via research funding and a revenue model based on sample provision. Several permissions were sought to ensure robust and informative linkage to relevant clinical information and recently the archive was added within the Lothian NRS BioResource[1]. Access to samples is obtained through application to a multi-disciplinary archive steering committee[2].

As a dynamic archive, it is a collection of collections and includes samples from women attending routine screening in addition to research collections associated with specific inclusion criteria. Currently, the archive contains over 45,000 samples (37,613 liquid based cytology, 8,231 nucleic acid extracts and 863 self-taken vaginal swabs). Samples are annotated with HPV and vaccination status, as well as pathology information. Quality assessment is performed regularly to assess best storage conditions for viable cells, DNA, RNA and protein.

Conclusion

To date, 51 applications have been approved for use, with an increase in applications over the last two years. Requests are associated with research into HPV epidemiology (5, 9.8%), new technologies for HPV detection (20, 39.2%), validation and assessment of HPV detection assays (17, 33.3%) and basic research into HPV (9, 17.6%). The applications have been both from United Kingdom (40, 78.4%) and international partners (11, 21.6%%); and 11 (21.6%) have involved commercial collaborations. The archive has been associated with several grants and peer reviewed publications[3] with outputs disseminated at national and international microbiology and oncology meetings. A recent challenge is the increasing and understandable demand that is made on nucleic acid quality and yield (from clinical samples) to reconcile with sophisticated molecular technologies that require long reads. Our intention is to maximise/optimise processing extraction and storage conditions to enhance quality.

References

In the nine years since its establishment, the Scottish HPV Archive has proved to be a valuable resource for researchers. Our aim is to further collaborate with the international community to: establish best practice for biobanking, determine what type of samples would support research optimally and consider joined-up options for funding/sustainability.

References

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00091

A Danish Clinical Cervical Cytology Biobank. Pilot studies of sample processing and quality

20. Diagnostic procedures / management

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Background / Objectives

Using liquid-based cytology usually only a smaller portion of the collected material is used for primary diagnostics (cytology and/or HPV testing.) The residual material is stored in either uncontrolled condition or discarded. For the purpose of future diagnostics and in order to continuously monitor and evaluate new screening methods and biomarkers, a cervical cytology biobank is very valuable. The objective of this study was to identify and evaluate an efficient workflow for establishing a cervical cytology biobank with high cell yield and high quality of the stored material.

Results

The biobank will consist of residual material from liquid-based cytology samples (ThinPrep, Hologic) collected from women participating in the national screening program for cervical cancer in the uptake area of Sygehus Lillebaelt, Denmark (approx. 50,000 samples/year).

The workflow shown in figure 1 is automated using the Freedom Evo 200 robot (Tecan), and information on samples and storage is administrated by the Labware LIMS system.

Cell yield was evaluated by measuring the amount of DNA in the original ThinPrep vial compared to the yield of DNA in the biobanked sample.

As an estimate of quality biobanked samples were examined by PCR with a 600 bp amplicon and with an NGS panel (TST15 panel, Illumina). In addition, imprint of a subset of samples have been compared before and after biobanking.

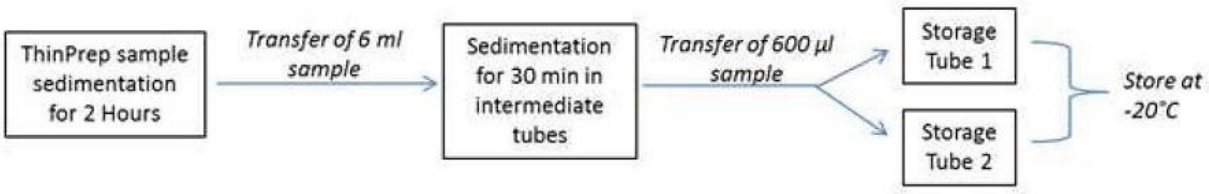
Conclusion

Based on 24 samples the DNA yield in storage tube 1+2 was on average 47% of the content in the primary tube. PCR results showed that 600 bp amplicons could be amplified for all samples, revealing high quality DNA. The DNA is also useful for NGS, as the analysis using the TST15 panel showed good quality parameters and high amplicon coverage.

A gynecological pathologist examined the imprint from samples before and after biobanking and no differences were observed, indicating that cells in the biobank are intact and could be used for analyses like IHC, FISH etc.

References

Using the presented workflow, a cytology biobank has just been initiated. Updated and further data on quality measurements of DNA, RNA and protein will be presented. The biobank holds great potential for future clinical purposes as wells as for research and quality assurance.



FC 02. Molecular markers 1

00084

TAME-SEQ: AN EFFICIENT SEQUENCING APPROACH TO CHARACTERISE HPV GENOMIC VARIABILITY AND CHROMOSOMAL INTEGRATION

01. Viral and molecular biology

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Background / Objectives

HPV genomic variability and chromosomal integration are important in the HPV-induced carcinogenic process. To uncover these genomic events in an HPV infection, we have developed an innovative and cost-effective sequencing approach named TaME-seq (tagmentation-assisted multiplex PCR enrichment sequencing). TaME-seq combines tagmentation and multiplex PCR enrichment for simultaneous analysis of HPV variation and chromosomal integration, and it can also be adapted to other viruses.

Results

For method validation, cell lines (n=4), plasmids (n=3), and HPV16, 18, 31, 33 and 45 positive clinical samples (n=21) were analysed. Samples were subjected to tagmentation using Nextera DNA library prep kit. Following tagmentation, target enrichment was performed by multiplex PCR using HPV primers and a combination of i7 index primers (adapted from Kozich et al., 2013) and i5 index primers from the Nextera index kit. Sequencing was performed on the Illumina MiSeq or Hiseq2500 platform. Data was analysed by an in-house bioinformatics pipeline.

Conclusion

Results showed deep HPV genome-wide sequencing coverage and high on-target read mapping. Chromosomal integration breakpoints and large deletions were identified in HPV positive cell lines and in one clinical sample. A high number of low frequency variants was observed throughout the HPV genome in all the samples.

References

In contrast to other approaches, TaME-seq proved to be highly efficient in HPV target enrichment, leading to reduced sequencing costs. The unique design of TaME-seq enables simultaneous analysis of HPV variation and chromosomal integration. Comprehensive studies on HPV intra-host variability generated during a persistent infection will improve our understanding of viral carcinogenesis. Efficient identification of both HPV variability and integration sites will be important for the study of HPV evolution and adaptability and may be an important tool for use in cervical cancer diagnostics.

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00095

HUMAN PAPILLOMAVIRUS TYPE 16 GENOMIC VARIATION IN WOMEN WITH SUBSEQUENT IN SITU OR INVASIVE CERVICAL CANCER: PROSPECTIVE POPULATION-BASED STUDY

02. Epidemiology and natural history

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Background / Objectives

HPV genomic variation may be involved in viral carcinogenesis.

Results

In a national register-based nested case-control study, we retrieved archival smears from baseline cytologically normal women who later developed cancer in situ (CIS), squamous cervical cancer (SCC) or remained free of disease. These smears were previously HPV-tested by PCR and HPV16 was the strongest risk factor. We now used the Illumina NextSeq platform to sequence HPV16 genomes in cervical smears from 242 women who later developed CIS/CIN3 (n=134), SCC (n=92) or remained healthy (n=16).

Conclusion

The median sequence depth per sample was high (11288x). For 218/242 samples (>90%), we covered ≥80% of the complete HPV16 genome with sequencing median depths of >200x. We identified a wide range of unique isolates and 343 novel SNPs across the 218 samples. Most women (97%) had HPV16 lineage A infection, with the sublineages being A1 (66.1%), A2 (28.9%) and A4 (1.8%), respectively. The least variable gene was the E7 (3.4% variability), where 170/204 case women (83%) displayed a fully conserved sequence. There were no obvious differences by disease outcome (CIS or SCC).

References

We found a high number of novel SNPs. The E7 gene was hypovariable both among women developing CIN3/CIS and SCC.

00414

CONCORDANCE OF HPV16 VARIANTS BETWEEN HETEROSEXUAL PARTNERS IN THE HITCH COHORT STUDY

02. Epidemiology and natural history

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Background / Objectives

Molecular variants from some phylogenetic branches of HPV16 are more likely to cause cervical lesions than others. Data on transmission dynamics of variants are lacking; we studied concordance of HPV16 variants within individuals and between sexually active couples.

Results

We used data from HITCH, a prospective cohort study of recently formed, heterosexual couples (women aged 18-24, men 18+) in Montreal between 2005 and 2013. Genital, oral, and hand samples were collected at clinic visits for up to two years. Samples were tested for HPV DNA by Linear Array genotyping PCR assays; HPV16-positive samples were analyzed by PCR sequencing using primers flanking a segment of the long control region. We conducted cross-sectional analyses of HPV16 variants at the same visit. Wilcoxon rank-sum tests were used to calculate differences in viral loads.

Conclusion

Of 674 samples positive for HPV16, we could study intratypic variation in 584 (86.6%) samples from 201 subjects. Invalid samples had a lower viral load than valid samples (P<0.001). We identified 33 variants. Most (n=176, 87.6%) HPV16-positive subjects had only one variant during HITCH; we identified a maximum of three variants in four participants. All but one (99.8%) sample contained one variant.

Within individuals, genital-hand concordance of HPV16 variants was significantly higher than by chance; the observed/expected (O/E) ratio was 6.6 (95% confidence interval (CI): 4.0-10.2) for women, and 7.8 (95%CI 4.3-13.1) for men. All men with a detected HPV16 variant in hand samples had the same variant in penile samples (n=14). In women, hand-to-genital concordance rate was 87.0% (n=25). Intra-individual oral-genital concordance was only increased in men (O/E ratio=4.2, 95%CI: 1.1-10.8), while oral-hand concordance for HPV16 variants was neither increased in men nor women.

Between sexual partners, genital-genital concordance of HPV16 variants was 60.8% (O/E ratio=4.4, 95%CI: 3.4-5.6). Hand-hand (48.6% concordance, O/E ratio=10.2, 95%CI: 4.7-19.4) and hand-genital (concordance range: 25.0%-86.7%; O/E ratios 6.8 (95%CI: 3.6-11.6) and 5.3 (95%CI: 2.9-8.9) for male hand-female genital and female hand-male genital concordance, respectively) variant concordance rates were increased. However, oral-genital, oral-hand, and oral-oral concordance between partners were comparable to concordance rates expected by chance (concordance range: 0.0%-42.9%).

References

Within young, sexually active, heterosexual couples included in HITCH, we found a high genital-genital, genital-hand, and hand-hand concordance of HPV16 variants. Concordance of HPV16 variants in the oral cavity and other body sites was low.

00494

CHARACTERIZATION OF T-CELL SURFACE MARKERS IN PERSISTENT HPV INFECTED MOTHERS AND THEIR CHILDREN

04. Immunology

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Background / Objectives

The host adaptive immune system plays a central role in preventing persistent HPV infections. Especially effectively functioning T-cells are important, but characterization of T-cell subpopulations between different HPV infection outcomes are not well known. The aim of this study was to characterize the T-cell surface markers associated with persistent genital and oral HPV16 infection among mothers' and their children in the Finnish Family HPV Study (FFHPV).

Results

FFHPV study was originally designed to clarify the dynamics of HPV transmission infections within regular 329 Finnish families. For this present study a subgroup of 42 mothers and their children (n=28) with a 14-year follow-up where evaluated according to the mothers' HPV infection outcome. The following groups of mothers and children were generated: 1) mothers who developed an incident CIN (mothers n=10, children n=10), 2) mothers who had a persistent oral HPV16 infection (mothers n=7, children n=7), 3) mothers who tested always oral HPV16 DNA negative (mothers n=5, children n=3) and 4) mothers who tested always genital HPV16 DNA negative (mothers n=20, children n=8). In addition to the lymphocyte stimulation test (LST) with fresh isolated peripheral blood mononuclear cells (PBMCs) to determine proliferation and cytokine production, the cryopreserved PBMCs were thawed and subjected to phenotypic flow cytometric analysis using antibodies directed to CD3,

CD4, CD8, CD25, CD27, CD38, CD45RA, CD45RO, CD57, CD69, CCR7 and HLA-DR.

Conclusion

The HPV16 E2 and E6 specific lymphocyte proliferation showed to be less common among persistent oral or genital HPV16 infected mothers than HPV16 negative mothers, while among children of HPV16 CIN mothers the specific proliferation was very common and offers possible protection for future HPV16 infections. The levels of IFN-γ ($p=0.014$) and IL-5 ($p=0.040$) was lower in mothers with oral HPV16 DNA positivity and the level of HPV16 E6-specific IL17 ($p=0.035$) was lower in mothers with CIN compared to controls. Analyses of the circulating T-cells, focusing on activation markers and memory markers, are currently ongoing and these results will be compared between the study groups.

References

HPV-specific responses detected in blood of mothers is related to those mothers who have a persistent infection and therefore merely a measure for the presence and visibility of the virus while detecting proliferating responses in their children provides a possible protection for future HPV infections. Characterization of the T-cell response by flow cytometry is expected to reveal T-cell subsets which are correlating with these different groups of mothers and their children.

00382

ASSOCIATION BETWEEN INTEGRATION OF HIGH-RISK HPV GENOMES, DETECTED BY MOLECULAR COMBING, AND THE SEVERITY AND/OR CLINICAL OUTCOME OF CERVICAL LESIONS.

08. HPV testing

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Background / Objectives

Background : Integration of the high-risk Human Papilloma Virus (HPV) in the cell genome is considered to be a key event in the development of cervical cancer and as one of its most important risk factor. Detecting HPV integration may therefore provide a useful marker for the identification of high-grade lesions and lesions at risk of progression. Molecular combing associated to specific Genomic Morse Code (GMC) is a powerful and innovative approach that allow accurate detection and quantification of integrated HPV sequence into the host genome.

Objectives : The aim of the EXPL-HPV-002 study is to evaluate the integration of 14 high-risk HPV as a biomarker of the severity and the progression of cervical lesions. Such a «triage biomarker» would help to reduce the number of unnecessary colposcopies, to avoid over-treatment of lesions that spontaneously regress and to better target the lesions requiring treatment.

Results

Methods : EXPL-HPV-002 is a prospective study conducted in 2 clinical sites in Czech republic. So far, 688 patients aged 25-65, referred to colposcopy after an abnormal Pap-smear, were enrolled in the study. Among them 60% were found HPV high-risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

The study is divided in 2 phases : (1) a transversal phase using data collected at first visit (colposcopy images +/- histology, pap-smear for HPV genotyping and molecular combing) to study the association between HPV integration status versus colposcopy and histology grades. (2) a longitudinal phase using data collected in follow-up visits : cytology at 6, 18 and 30 months and colposcopy +/- histology at 12, 24 and 36

months. A pap-smear collected at 12, 24 and 36 months allows to perform genotyping and molecular combing. HPV integration status is analyzed in comparison with the evolution of lesions, viral clearance and HPV genotype.

HPV genotyping and molecular combing are performed in central laboratories, histology data are reviewed by central reading.

References

Conclusions : The transversal phase of the clinical study is achieved while the longitudinal phase data collection is still ongoing. Results of the diagnostic phase show that the HPV integration monitored by Genomic Vision’s technology is a reliable biomarker that can significantly differentiate normal subjects from women with a risk to develop precancerous lesions or cancer. Preliminary results on prognostic value of the test will be presented as well.

00002

CKAP2 EXPRESSION SERVES AS A NOVEL POOR PROGNOSTIC FACTOR IN CERVICAL CARCINOMA

12. Molecular markers

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Background / Objectives

To study the expression level of cytoskeleton-associated protein 2 (CKAP2) in cervical cancer tissues, and to analyze the relationship between abnormal expression of CKAP2 and clinicopathological factors and prognosis of cervical cancer.

Results

We first screened CKAP2 as a new candidate oncogene in two independent data sets (TCGA and gse27678). Immunohistochemistry, RT-PCR and Western blot were then used to verify the expression of CKAP2 in cervical cancer tissues, which association with clinical features was further analyzed by statistical method.

Conclusion

The expression of CKAP2 was significantly upregulated in cervical carcinoma tissues when compared with adjacent normal counterparts. Then clinical characteristics of human cervical carcinoma tissues were further classified into the high-CKAP2 group (n= 125) and low-CKAP2 group (n= 122) using the median expression value of CKAP2 as the cutoff point. The results showed that increased CKAP2 expression was significantly correlated with age, FIGO stage, lymph node metastasis, recurrence and tumor size, but not other clinical characteristics. The survival time of cervical carcinoma patients showed that patients with under-expressed CKAP2 expression notably lived longer than patients with over-expressed CKAP2 expression. We next performed univariate and multivariate analysis of prognostic factors for overall survival with the Cox regression model. We identified three prognostic factors, including FIGO stage, Lymph node metastasis and CKAP2 expression, can served as independent prognostic factors for poor overall survival.

References

In conclusion, our findings demonstrated that CKAP2 was overexpressed and served as an independent biomarker for poor prognosis in cervical carcinoma.

00243

CO-EXPRESSION OF HPV E6, E7 MRNA AND PD-L1 IN CERVICAL CYTOLOGY SAMPLES

12. Molecular markers

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Background / Objectives

HPV infection in most women is transient and clears over time. For others, the virus is persistent and can lead to pre-cancerous lesions and subsequently cervical 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 immuno-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, cell cycle analysis, and PD-L1 cell surface staining on epithelial cells in liquid-based cervical cytology specimens.

Results

Forty-six residual cervical cytology specimens were obtained for this study: 25 HPV DNA-, 12 LSIL HPV DNA+, and 9 HSIL HPV DNA+. Samples underwent in-situ hybridization with E6,E7 mRNA probes and a cell cycle dye. Anti-PD-L1 antibody was added following in-situ hybridization. Samples were collected on a Beckman Coulter CytoFLEX. Samples were deemed positive or negative for E6,E7 and Post G1 expression by a dual cut-off of 3.15%. PD-L1 expression was determined based on a cut-off of 2%.

Conclusion

Sample	Positive %E6,E7 and Post G1	Positive %PD-L1	% Dual E6,E7 and PD-L1
HPV DNA-	24% (6 of 25)	24% (6 of 25)	83% (5 of 6)
LSIL	50% (6 of 12)	25% (3 of 12)	50% (3 of 6)
HSIL	33% (3 of 9)	11% (1 of 9)	33% (1 of 3)

References

In this study we show dual E6,E7 and PD-L1 expression on the same sample. HPV and PD-L1 expression on cell by cell basis is not currently available in a single test by any other method. It appears PD-L1 expression decreases in high grade lesions indicative of immune surveillance which could support therapeutic options.

00254

The inverse relation between expression of pan-HPV E4 and methylation markers FAM19A4/miR124-2 in the identification of productive and transforming cervical intraepithelial neoplasia

12. Molecular markers

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Background / Objectives

To identify productive and transforming cervical intraepithelial neoplasia using HPV E4 and p16 immunohistochemistry; to determine the methylation positivity as detected on cervical smear, of E4/p16-identified transforming lesions.

Results

Women whose inclusion smear was tested for FAM19A4/miR124-2 hypermethylation and who had a worst lesion of CIN1-3 detected on biopsy were selected from a prospective follow-up study (EVAH study). Biopsies were cut and stained for H/E, E4 and $p16^{INK4a}$. Lesions of which the diagnosis on the original section differed from the diagnosis on the new section were excluded; 188 remained. Women with initial E4 positive and E4 negative lesions were compared for methylation status in the inclusion smear and grade of p16 stain of the initial worst lesion.

Conclusion

179 biopsies were included: 58 CIN1, 78 CIN2 and 43 CIN3. 44.8% of CIN1, 19.2% of CIN2 and 4.7% of CIN3 lesions were E4 positive. A cervical smear positive for FAM19A4/miR124-2 was found in 22.4% of women with CIN1, 43.6% CIN2 and 72.1% CIN3. We found a significantly higher proportion of E4 positivity of the worst lesion present in women with a methylation- smear (30.7% E4+) compared to a methylation+ smear (15.4% E4+) ($p=0.017$, $r=-0.178$). 69.8% of E4+ lesions showed p16 in ≥2/3 of the epithelium.

References

E4 positive lesions are lesions in the productive phase of the HPV lifecycle and most likely relatively recent infections as indicated by the negative correlation with methylation status. Extensive diffuse p16 expression did not indicate a non-productive, fully transformed lesion.

00338

INTER-LABORATORY REPRODUCIBILITY OF THE P16INK4A/KI-67 DUAL STAINING IN HPV POSITIVE WOMEN FROM THE NTCC2 STUDY

12. Molecular markers

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Background / Objectives

New Technologies for Cervical Cancer 2 (NTCC2) is a large randomized clinical trial within organized cervical screening programs in Italy using HPV-DNA as primary screening test. The aim of NTCC2 is the evaluation of new biomarkers as triage test of HPV-DNA positivity, in comparison to cytology. In particular, we are evaluating Aptima HPV Assay (Hologic) for HPV E6-E7 mRNA, and CINTec PLUS Assay (Roche Diagnostics) for the immunocytochemical dual-staining of p16ink4a and Ki-67 proteins. In a previous study the p16ink4a/Ki-67 dual staining showed a good reproducibility between readers from nine different laboratories on selected immunostained slides, confirming its robustness, which is a necessary requisite for introduction in cervical cancer screening (1). In this study we assessed the inter-laboratory reproducibility of the test interpretation among the samples enrolled in the NTCC2 study.

Results

ThinPrep liquid based cytology slides from baseline HPV-DNA positive women, were immunostained by CINtec® PLUS Assay in four centres and were interpreted in seven different centres involved in cervical cancer screening and/or in cervical cancer research. Immunostaining results were classified as positive (at least one double stained cell), negative, or inadequate. Each immunostained slide was analyzed and scored independently by three different laboratories giving a total of 4027 reports. To evaluate inter-laboratory interpretation reproducibility, kappa values for multiple raters were reported for the overall agreement and ninety-five percent confidence intervals (95%CI) were calculated, using the bootstrap method with bias correction.

Conclusion

Overall, 454 out of the 4027 reports were inadequate (11.2%), mainly because of scant cellularity or staining decay. The overall concordance for adequacy was poor (kappa value 0.155, 95%CI: 0.106-0.213). However, considering only the consecutive first and second interpretations, the kappa value increased to 0.425 (95%CI 0.325-0.526). When we took into consideration only the 3573 evaluable reports, we observed 1261 positive (35.3%) and 2312 negative readings (64.7%), with a good concordance for positivity (kappa value 0.623, 95%CI 0.586-0.665). Also in this case, considering only the first and the second reports the kappa value rose to 0.746 (95%CI 0.705-0.788).

References

The dual-staining for p16ink4a and Ki-67 showed a good reproducibility for positivity, which is a necessary prerequisite for adoption as a triage test in cervical cancer screening programs with HPV-DNA as primary test.

References

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00456

CERVICAL INTRAEPITHELIAL NEOPLASIA AND CERVICAL CANCER: A GENOME WIDE ASSOCIATION STUDY (GWAS) OF UK BIOBANK AND NORTHERN FINNISH BIRTH COHORTS (NFBC66)

12. Molecular markers

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Background / Objectives

Persistent infection with high-risk human papillomavirus (HPV) is causally associated with cervical cancer. However, only ~1% of women with HPV infection progress to cervical neoplasia (CIN). It is estimated that genetic heritability may explain 25-30% of total variation in liability for cervical cancer. Common genetic variants have been detected in HLA (Human Leukocyte Antigen) regions responsible for the immune response, but this is not well understood. We conducted a genome-wide association study, in two cohorts, to identify underlying genetic risk variants which might predispose to CIN and cervical cancer.

Results

Using Northern Finland Birth Cohort 1966 (NFBC66) and Finnish nationwide registers we identified 365 women with CIN/cervical cancer and 1678 controls without a history of any cytological abnormalities. Using UK Biobank data and United Kingdom (UK) national cancer registries we identified 6378 women with CIN3/cervical cancer and 198,441 controls, this represents the largest cervical cancer GWAS to date. We conducted genome wide analyses for CIN or cervical cancer first in NFBC66 followed by UK Biobank.

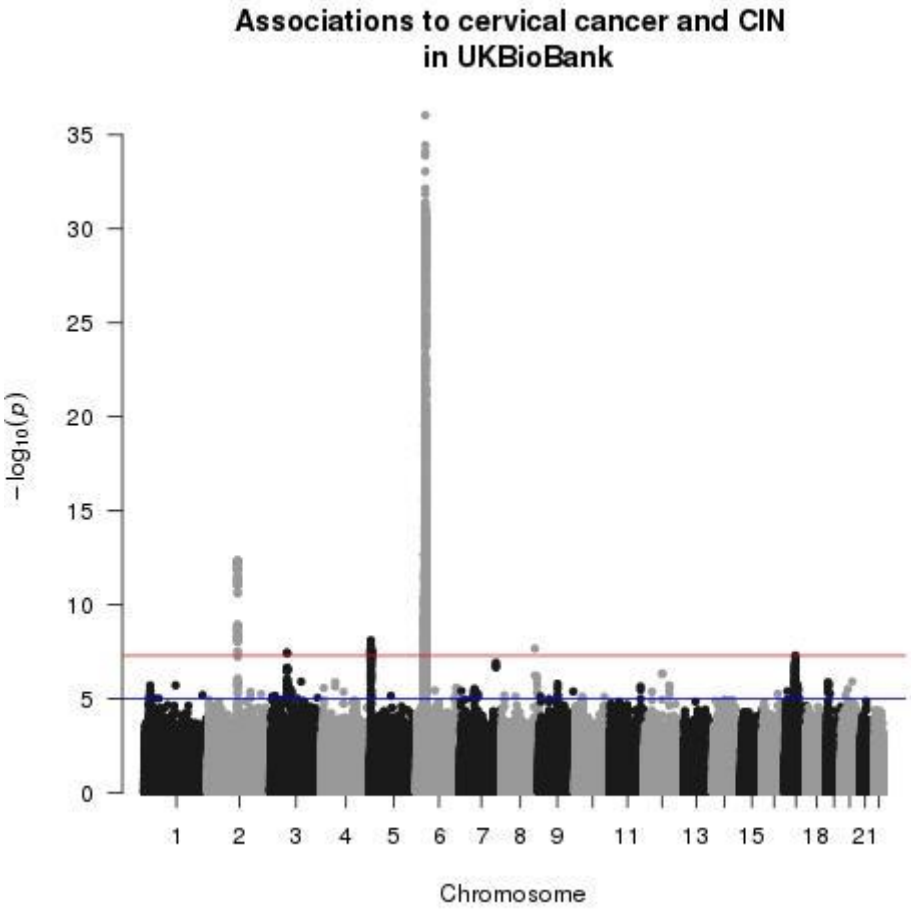
Conclusion

In the NFBC66 cohort we identified SNPs ($p<5x10E-8$) associated with increased risk of CIN or cervical cancer. Two of the top variants were associated with three protein-

coding genes at the same locus: PIBF1, BORA and MZT1, all with roles in mitotic cell division and/or cancer development. In the first UK Biobank iteration we have identified potential SNPs (p<5x10E-8) associated with CIN3/cervical cancer, with a large number of significant loci residing within Chromosomes 2 and 6 (Figure 1). Independent loci in the Major Histocompatibility Complex (MHC) region at 6p21.3 were associated with CIN3/cervical cancer, including loci adjacent to the MHC class 1 polypeptide-related sequence A gene (MICA) and HLA-DRB1, which replicates previously reported associations from published GWAS.

References

We observed genetic variants significantly associated with CIN or cervical cancer in both cohorts. Loci within the MHC may affect susceptibility to development of CIN3/cervical cancer through altered immune responses. We will next undertake fine-mapping within the UK Biobank cohort, to determine replication of NFBC66 findings and further classify any novel causal variants that may explain the estimated genetic susceptibility to cervical cancer.



00539

BIOMARKER DISCOVERY FOR IN VIVO IMAGING OF CERVICAL PRECANCERS

12. Molecular markers

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Background / Objectives

Due to the low specificity of HPV DNA testing, triage testing for positively screened women is required to reduce overtreatment harms, but current 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. We used a gene-expression based biomarker discovery approach for development of in vivo imaging markers.

Results

The Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED) recruited women referred for colposcopy with abnormal screening results. Gene expression levels were determined in mRNA microarrays of SUCCEED tissue from 128 patients at all stages of progression to cervical cancer, and differential expression of genes compared between cervical intraepithelial neoplasia grade 3 (CIN3) and combined CIN1/normal tissues. Candidate biomarkers with nominally significant p-values (<0.01) and higher expression in CIN3 (fold-change >2) were investigated for membrane localization and enzymatic activity. Initial validation of the top candidate genes through immunofluorescence staining of cervix tissue microarrays was followed by immunohistochemical validation of the most promising candidates in full tissue slides from SUCCEED.

Conclusion

Using these criteria, we found 48 potentially plasma membrane-bound proteins that could be amenable to in vivo staining and visualization. Candidates were evaluated for likelihood of membrane localization, enzymatic activity that would facilitate the development of an in vivo imaging probe, availability of validated antibodies, and evidence of cancer-associated alterations in protein expression or function. Candidates were prioritized, and 11 high priority proteins were stained in cervix tissue microarrays that included six cancers and six adjacent normal tissue cores. MUC4 exhibited strong membrane staining in all the cancers and negative to weak staining in normal epithelium samples. MUC1 exhibited low to moderate membrane staining in normal epithelium and moderate to strong staining in cancer. These two candidates were selected for further validation through immunohistochemical staining of SUCCEED tissue slides using conventional histology evaluation and automated image analysis. This effort 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.

References

The discovery of membrane biomarkers of cervical cancer and precancerous lesions may enable the development of specific, sensitive, low cost in vivo detection tests for prevalent precancers.

FC 03. Vulvar and penile HPV diseases

00036

PREVALENCE OF HPV IN FRESH TISSUE OF PENILE CANCER

02. Epidemiology and natural history

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Background / Objectives

The prevalence of HPV in penile cancer have been shown to be around 47 %, ranging between 24% and 82 % (1, 2). Analysis has mostly been performed on formalin embedded tissue, except in 74 cases of fresh tissue (1). The large variation in HPV-prevalence is most likely due to different histological type of tumors and different methods for HPV analysis. Our aim was to determine the prevalence of HPV in fresh tissue of invasive penile cancer cases and from nonmalignant penile controls.

Results

Fresh tissue from consecutive invasive penile cancer cases operated in Skane University Hospital from June 22, 2015 through June 1st 2018 was biopsied immediately after arrival at the Department of Pathology. Controls were men circumcised of nonmalignant reasons, with biopsies taken in the operation room. All penile cancer cases and all controls filled in a survey regarding general diseases, medication, former symptoms, diseases and surgical procedures on penis and number of sexual partners. Each 2 mm biopsy was put in RNAlater (Ambion) and transferred to 1 mL GITS-solution (4M guanidinium thiocyanate, 22mM NaCitrate and 5% Sarcosyl (N-Lauroylsarcosine sodium salt) and 1% mercaptoethanol) and incubated at room temperature overnight. Then DNA was extracted with the Total NA-kit (Roche, Stockholm, Sweden) using MagNA Pure LC (200 uL input and 100 uL output). Sample adequacy was assessed by testing 5 uL of the sample for the human beta globin gene with a real-time PCR. Simultaneous identification of 40 genital HPV types were carried out by modified general primer polymerase chain reaction (MGP-PCR) and subsequent Luminex analysis in a 25 uL reaction, containing 5 uL of extracted material. The Luminex assay included probes for HPV types 6, 11, 16, 18,

26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 85, 86, 87, 89, 90, 91 and 114.

Conclusion

Hitherto, 83 men with invasive penile cancer have been included and 214 controls without penile cancer. Mean age of men with invasive penile cancer were 68.8 years (range 28-87 years) and for controls 46 years (range 19-90 years). Of penile cancer cases, 42.2 % (35/83) had HPV in the tumour. High risk HPV types were found in 41.0 % (34/83), where HPV 16 was present among 32.5 % (27/83). In 6.0 % (5/83) multiple HPV types existed. Among the controls, 13.6 % (29/214) had HPV in the circumcised tissue. High risk HPV types were found in 6.1 % (13/214), where HPV 16 was present 1.4 % (3/214). In 3.7 % (8/214) of controls, multiple HPV types were present.

References

HPV is more common in invasive penile cancer than in non-malignant controls ($P<0.0001$). HPV 16 is the predominant HPV type.

References

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00367

PREVALENCE AND DETERMINANTS OF HUMAN PAPILLOMAVIRUS IN MEN AND TRANSGENDER WOMEN WHO ARE SEX WORKERS: SWEETIE STUDY

02. Epidemiology and natural history

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Background / Objectives

Men and transgender women (TGW) who are sex workers are an exposed and vulnerable population for Sexually Transmitted Infections (STI), of difficult access, resulting in a lack of information about prevalence and determinants of STIs in general and Human papillomavirus (HPV) in particular. To estimate the anogenital and oral HPV prevalence and determinants in men and TGW who report to be sex workers.

Results

Men and TGW aged ≥18 residents in Barcelona are enrolled in a cross-sectional study conducted in collaboration with local non-governmental organizations, STOP SIDA. Demographic and behavioral characteristics are assessed by questionnaire, and anal, perianal, penis, urine and oral samples were collected for HPV testing and genotyping.

Conclusion

From a total of 37 participants, 25 were TGW (recruitment is currently ongoing). Average age was 33 years and most participants were foreigners (97.4%), mainly from South America. 23% reported to be HIV-positive and 35.1% had another active STI. The prevalence of HPV was 91.6% in anal, 12.9% in oral and 6% in urine samples. Many participants were unfamiliar with HPV vaccination, but 87.1% expressed positive attitudes for vaccination. We estimate to collect a total of 450 samples from 90 participants until the end of the recruitment phase (July, 2018). HPV results from all patients and all locations will be presented at the IPVC 2018.

References

The prevalence of HPV infections is higher in this population than general population, mainly in anal and oral sites. Specific HPV vaccine programs addressed to this population should be considered.

References

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00361

MEN, THE FORGOTTEN VICTIMS FOR HPV DIAGNOSIS

08. HPV testing

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Background / Objectives

In men, high risk (hr) types can induce penile or rectal intraepithelial neoplasias or penile cancer, while low risk (lr) HPV types, may cause genital warts or atypical genital or perigenital lesions. Aim of the retrospective study in men was to evaluate the results of lr- and hr HPV genotyping in male samples, collected at or sent to the Outpatients Centre for STI Diagnosis in Vienna.

Results

Out of 7032 patients tested for HPV between January and June 2018, 746 (10.6%) samples were collected mainly from the penile (46%), perigenital (26%), and anorectal (10%) area, respectivley, from male individuals with an average of 37 years. Patients were either referred to the Outpatients centre for HPV diagnosis or samples were sent and examined for the presence of HPV high- and low-risk genotypes by using the PapilloCheck®. This is a microarray-based assay for the detection and identification of 18 hr HPV and 6 lr HPV types, based on the detection, amplification, and genotyping of a 350pb fragment of the viral E1 gene.

Conclusion

The main indications for testing HPV, was the presence of genital warts (37.4%), a suspicious HPV infection (38.1%), and partner control (4.6%). Of the 420 HPV positve samples (57%), 46,7% were lrHPV, 25.2% hrHPV, and 28,1% both, lr and hrHPV positive.

The most prevalent lr-genotypes were HPV 6 (24.6%), 11 (4%) and 42 (8.8%), respectively, and hr-genotypes were HPV 16 (10.3%), 51 (6.1%) and 56 (6%), respectively. In men with genital warts, 78% were HPV positive including lrHPV only (47.7%), hr HPV only (12,5%) or a mixed infection (17.8%). Serotyping showed HPV6 as the most prevalent lr genotype (37.8%) followed by other lr-HPV (24%). Among hr genotypes, HPV 16 (9.5%) was the most common one. While all men after treatment of genital warts were lr HPV negative, still 14.3% were hr HPV positive. In

men, tested due to contact tracing, either lr HPV or hr HPV were positive in 15.2%, respectively, and in 23.9% harboring both, lr and hr HPV.

References

In men with genital warts the high prevalence of HPV 6 was confirmed. However, also hr HPV, or a coinfection with hr HPV were detected in about one third of men with genital warts. In almost half of the men with suspicious HPV infection HPV diagnosis could be confirmed. The high percentage of hr HPV types either present as single or mixed infections should be considered for further controls in men and demonstrates the need for HPV diagnosis in men.

00171

DNA METHYLATION MARKERS FOR RISK STRATIFICATION OF VULVAR INTRAEPITHELIAL NEOPLASIA

24. Vulvar diseases and neoplasia

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Background / Objectives

High-grade vulvar intraepithelial neoplasia (VIN) is the precancerous state of vulvar squamous cell carcinoma (VSCC). Only a minority of VINs progress to cancer, indicating a heterogeneous disease. 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. In this study we assessed the potential value of DNA methylation markers for risk stratification of VIN.

Results

A series of 200 FFPE specimens, including normal vulva samples (controls), VIN cases and VSCC cases were included in this study. Of the VIN cases, VIN with associated VSCC (VIN with VSCC, i.e. VIN adjacent to VSCC or development of VSCC in the follow-up) and VIN without associated VSCC (VIN without VSCC, i.e. VIN without concurrent VSCC and without development of VSCC during at least ten years of follow-up) were included. Samples were tested for p16INK4a immunohistochemistry and for HPV DNA to define the HPV status. Multiplexed quantitative methylation-specific PCR assays were performed on nine candidate methylation markers to analyse differential methylation.

Conclusion

In both HPV-negative and -positive cases, methylation levels were found to be increased in both VSCC and VIN with VSCC, compared to controls and VIN without VSCC. Comparison of HPV-positive VIN without VSCC and VIN with VSCC, yielded a number of methylation markers with an area under the curve (AUC) > 0.8.

References

Our results show that vulvar carcinogenesis is associated with increased methylation of (candidate) tumour suppressor genes. We identified multiple methylation markers for risk stratification of VIN lesions, which are promising for tailored management in affected women.

00301

HISTOLOGICAL CHARACTERISTICS AND OVERALL SURVIVAL OF HPV ASSOCIATED AND INDEPENDENT SQUAMOUS CELL CARCINOMA OF THE VULVA: A RETROSPECTIVE STUDY

24. Vulvar diseases and neoplasia

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Background / Objectives

The presence or absence of HPV separates vulvar squamous cell carcinoma (VSCC) into two distinct molecular and clinicopathological entities. Studies on histological and survival characteristics present discordant results. We assessed the impact of HPV presence in the histological characteristics and the overall survival (OS) in a cohort of VSCC.

Results

We report 93 cases of VSCC with HPV status, clinical, histological and prognosis data diagnosed over a period of 14 years (2002 to 2016). HPV DNA detection was done using SPF-10 PCR/ DEIA/LIPA v2 system. Kaplan-Meier estimator and multivariable Cox regression analysis controlling for FIGO stage and age were used.

Conclusion

The median age was 74 years (range 28-96). Patients with HPV associated tumours were older (median 78 vs 71). The mean follow-up time in this cohort was 3.6 years (range <1 to 11.4 years). HPV status was determined in all, 64 HPV-negative, 29 HPV-positive, of which 11 HPV16-positive. Tumours were histologically classified as keratinizing (78%) and nonkeratinizing (14%), basaloid (5%) and hybrid (2%). 55% of the tumours were moderately differentiated, the mean tumour thickness was 34 mm (range 1-121mm). Lymph node metastasis was present in 40% of the cases and the mean lymph node metastasis size was 10.3 mm (range 0.4-39mm). FIGO stage was I in 47%, II in 10% and III in 43% of the cases. Patients were initially treated with surgery (46%), radiotherapy (45%) and chemotherapy (9%) and 37% of the patients died of the disease. HPV associated tumours were more likely to have koilocytotic

change (p<0.01). The invasive front, inflammatory infiltrate, lymphovascular space invasion, perineural invasion, positive lymph nodes and positive margins for invasive tumour were not significantly different between the two groups. No differences were found between HPV-positive and -negative tumours regarding OS (hazard ratio (HR) =1.08, 95% CI=1.21-4,17, 0.56-2.06 p=0.82). Patients who underwent surgery had superior OS (HR=0.51, 95% CI=0.26-0.99 p=0.04) and lymph node metastasis size ≥ 5mm was associated with a statistically significant inferior OS (HR=1.88, 95% CI=1.22-2.92 p=0.004).

References

Although HPV associated tumours were more likely to have koilocytotic-like change, histological criteria did not allow differentiation between HPV associated and independent VSCC. No differences in survival were observed between HPV -positive and -negative tumours. Patients who underwent surgery had a superior OS compared to other treatments and in patients with lymph nodes metastasis ≥ 5mm the OS was inferior.

00308

VULVAR INTRAEPITHELIAL NEOPLASIA: INCIDENCE AND LONG TERM RISK OF VULVAR SQUAMOUS CELL CARCINOMA

24. Vulvar diseases and neoplasia

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Background / Objectives

Given the heterogeneity of vulvar carcinogenesis, the incidence of vulvar intraepithelial neoplasia (VIN) and the subsequent cancer risk is ambiguous. Human Papillomavirus (HPV) associated VIN accounts for more than 90% of all high-grade VIN, while HPV-induced vulvar squamous cell carcinomas (VSCC) only account for 25% of all VSCCs. To optimize monitoring and clinical care of women with VIN, this study aimed to obtain knowledge about both the incidence of VIN and the subsequent cancer risk in affected women.

Results

The PALGA database, the Dutch Pathology Registry, enabled us to obtain long-term follow-up data (up to 2018) from a large historical cohort of women with VSCC, VIN and lichen sclerosus (LS) diagnosed between 1991 and 2011.

Conclusion

: In our cohort, 1,147 women were diagnosed with VIN with an incidence rate of 3.8 cases per 100,000 woman-years. Between 1991 and 2011 the incidence of VIN increased with 67%. Although most women diagnosed with VIN were between 35 and 55 years of age, the incidence rate of VIN peaked in women ≥ 70 years. This peak at higher age is explained by the fact that the presence of concurrent VIN and VSCC increases with age. The cumulative incidence of VSCC in women with VIN was 13.8% after a follow-up period up to 27.5 years. The cancer risk in women with VIN was significantly higher in women with LS compared to women without LS (i.e.

10-years cumulative incidence of VSCC 36.7% in women with LS compared to 8.3% in women without LS, $p < 0.001$) . Subgroup analyses in women with VIN tested for HPV showed that women with HPV positive VIN had a significant lower cancer risk than those who had HPV negative VIN (10-years cumulative incidence of VSCC 9.0% in the HPV positive group compared to 30.4% in the HPV negative group, $p = 0.012$). The cancer risk in women with VIN increased with age ($p < 0.001$).

References

Our findings on a large cohort of women with VIN with long-term follow up support the different routes in vulvar carcinogenesis, with a favourable course in women with HPV-related and LS independent VIN. Because of the increased cancer risk in women with VIN diagnosed at a higher age and in women with HPV negative or LS associated VIN, intensified clinical care is needed in these patient groups.

00444

Why is it important to keep follow-up – a case-report of HPV 16 infection

24. Vulvar diseases and neoplasia

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Background / Objectives

Human papillomavirus (HPV) is the most common sexually transmitted infection. It is well established the relationship between high risk HPV (mostly 16 and 18) and the genesis of cervical cancer. Both primary (vaccine) and secondary prevention (screening with cytology and HPV test) are recommended.

Cervical lesions are the most common but, when the virus is identified, it is important to keep in mind the risk of lesion in other locations.

Results

Case report.

Conclusion

Female, 44 years-old, 1G 1Cesarian. Past medical history: smoker, diabetes mellitus 2 and hypothyroidism. No history of HPV vaccine.

She was referred in 2011 to a gynaecological clinic due to ASC-US result at pap smear. Both colposcopy and vaginoscopy were performed with biopsies: CIN1 and VAIN2. Cervical conization revealed a CIN1.

At the 6 month exam, she had a LSIL cytology, then performing colposcopy with biopsy: CIN 2 and VAIN2. HPV 16 positive.

Kept the follow-up according to Portuguese guidelines, having a NILM cytology, normal colposcopy and negative HPV test for three years.

Four years after the first referral she had a LSIL cytology, HPV 16 positive with normal colposcopy. One year after that, vulvar and vaginal lesions were identified and biopsied - vulvar dysplastic lesion and vagina without dysplasia.

She kept the follow-up at the gynaecological clinic, and seven years after the first appointment (May, 2018) she had a NILM cervical citology, HPV 16 positive and normal colposcopy. Vulvoscopy showed leucoplastic lesions of the posterior third of the large lips, acetowhite lesion with mosaic on the large right lip and on the inner side of the small right lip. Biopsies were performed - VIN 3 usual type (HSIL of the vulva).

A bilateral vulvectomy was performed, with excision of a perineal and a peri-anal lesion (which were not present at the pre-op exam). The anatomopathological examination revealed: High-grade intra-epithelial (condylomatous-u-VIN3) lesion bilaterally on the small and large lips and on the perineum. The peri-anal lesion was a high-grade condylomatous anal intraepithelial lesion (severe dysplasia AIN-3).

The postoperative period had no relevant intercurrences, with favorable healing. The patient remains under surveillance at the gynecology clinic.

References

This case highlights the pathogenesis of the HPV virus (high-risk HPV 16) and host susceptibility, as well as the effects and lesions at levels of the different locations, cervix, vagina, vulva and anus. A correct follow-up allows for diagnosis and treatment of the lesions in earlier stages, however there is an important degree of morbidity associated, which is why primary prevention has a paramount role.

00457

NIPPLE DERMOSCOPY FINDINGS POSSIBLY ASSOCIATED WITH HUMAN PAPILLOMA VIRUS (HPV) AND BREAST CANCER

28. HPV and associated skin diseases

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Background / Objectives

Many breast diseases start on the nipple. Some morbidity that affects the ductal system may show typical lesions on the nipple before clinical detection. In the last twenty years, papers have discussed HPV on the breast cellular cycle, and detected the HPV in breast neoplasia. Recently in the Federal University of Ceara (UFC), the relation between HPV and Breast Cancer (BC) is being studied and HPV DNA was found in 49.5% of patients with BC. Along with other publications on this subject we can infer that HPV can participate in breast carcinogenesis although the cause-effect is not characterized. There is much information about skin and mucosal lesions such as warts, mucosal pigmentation, and vascular alteration mainly in the cervix uteri that are associated to HPV. In January 2018, after authorization by the Ethics Committee of the UFC Hospital we have been researching dermoscopy images to identify findings of HPV on the nipples of patients with BC.

Results

Ten patients after biopsy and diagnosis of ductal carcinoma without clinical signs of nipple-areolar complex infiltration were studied with a dermoscopy guided 2mm punch biopsy followed by Polymerase Chain Reaction (PCR). HPV detection and genotyping were performed through the multiplex nested PCR technique, through a set of primers that amplify the HPV E6/E7 consensus region. HPV genotyping amplified the E6/E7 region of the genome, followed by region-specific amplification for each viral type. Ten types of HPV were investigated: 6/11, 16, 18, 31, 33, 45, 52, 56, and 58. An iPhone 7 adapted with a 10-20x magnification handyscope was used to perform the dermoscopy. The study was approved by the Ethic Committee of Federal University of Ceara

Conclusion

In all patients with breast cancer HPV positive, some nipple findings were observed. The resulting images demonstrate: increased vascularization, micro papillary lesions, small pigmentation changes, white patches and inflammatory lesions, which are frequently associated with HPV in colposcopy findings in HPV induced lesions of the cervix.

References

While some authors have used dermoscopy to identify other diseases on the nipple, such as Paget's Disease and melanomas, none have investigated visually identifiable lesions on the nipple associated with HPV. It is still unknown how HPV reaches the ductal systems, and it is possible that it does so through the nipple. If this is confirmed, a significant step can be made towards identifying a risk of BC. The possibility of a non-invasive method collaborating to the diagnosis of early BC is definitely helpful.

00305

TRENDS IN INCIDENCE, MORTALITY AND SURVIVAL OF PENILE SQUAMOUS CELL CARCINOMA IN NORWAY 1956-2015

36. Public health

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Background / Objectives

To examine trends in incidence, mortality and survival of penile squamous cell carcinoma (SCC) in Norway over 60 years.

Results

Data on all cases of penile cancer diagnosed in Norway during 1956-2015 was obtained from the Cancer Registry of Norway. Trends in age-standardised rates of penile SCC incidence, mortality and 5-year relative survival were assessed by the annual percentage change statistic and joinpoint regression

Conclusion

A total of 1596 penile cancer cases were diagnosed during 1956-2015, among which 1474 (92.4%) were SCC. During 2011-2015, the age-standardised incidence and mortality of penile SCC were 0.91 (95% confidence interval (CI): 0.78;1.05) and 0.50 (0.42;0.60) per 100,000, respectively, and the 5-year relative survival was 61.6% (41.9;76.4). The incidence of SCC increased during 1956-2015, with an average annual percentage change (AAPC) of 0.80% (0.46;1.15). The increase was strongest among men diagnosed at a relatively early age (age<=64 years; AAPC: 1.47% (0.90;2.05)). Mortality also increased over the study period (AAPC: 0.47% (0.10;0.85)), whereas 5-year relative survival did not change (AAPC: 0.08% (-0.19; 0.36)).

References

We conclude that the incidence of penile SCC has increased at a moderate and constant rate during 1956-2015, and that the most consistent increase occurred among younger men. Mortality also increased during the study period. However, survival did not change, thus changes in diagnostics and treatment had little impact

on survival from penile SCC. Since a substantial proportion of penile SCC is caused by human papillomavirus (HPV), the incidence increase may in part be attributed to increased exposure to HPV in the population.

FC 04. Vaccines 1: Male vaccines

00611

HUMAN PAPILLOMAVIRUS (HPV) SEROPREVALENCE AND ANOGENITAL HPV DETECTION AMONG HIV-NEGATIVE MEN WHO HAVE SEX WITH MEN (MSM)

02. Epidemiology and natural history

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Background / Objectives

Although seroprevalence can be used as a crude estimate of cumulative HPV exposure in a population, there are relatively few studies of type-specific HPV seroprevalence in males. We studied HPV seropositivity and anogenital detection at baseline in 602 MSM 17-27 years old participating in a multinational clinical trial of the quadrivalent HPV vaccine.

Results

A highly specific and sensitive competitive luminescence immunoassay (cLIA) was used to measure baseline seropositivity for the HPV types targeted by the 9-valent (9v) vaccine (6/11/16/18/31/33/45/52/58). Intra-anal, scrotal, perineal/perianal, and penile (“anogenital”) swabs were collected at baseline and analyzed for 14 HPV types, including the 9v vaccine types.

Conclusion

At baseline, 228 MSM (38%) had HPV detection of any 9vHPV vaccine type, of whom 41% were seropositive to the same HPV type and 64% were seropositive to any 9vHPV type. Seropositivity concordant with the same HPV type was: HPV6 (56%), HPV11 (35%), HPV16 (36%), HPV18 (23%), HPV31 (27%), HPV33 (11%), HPV45 (11%), HPV52 (17%), HPV58 (20%). HPV type concordance between

anogenital swab and seropositivity varied by swab anatomic location. Intra-anal [46% (38.3-52.7) and peri-anal / perineal swabs [47% (38.3-55.4) had higher concordance among seropositivity and anogenital detection of any of the 9vHPV types as compared to penile [31% (20.8-42.2)] or scrotal swabs [35% (23.5-47.6)]. In a sub-analysis of all 335 trial MSM from the US, EU, and Canada without HPV detection at baseline, 34% were seropositive for any 9vHPV type.

References

Young MSM had evidence of past HPV exposure, even without anogenital HPV detection. Approximately 2/3 of MSM with current anogenital HPV detection were seropositive to any 9vHPV type. HPV exposure in young MSM was common, emphasizing the need to vaccinate prior to sexual debut.

00612

HUMAN PAPILLOMAVIRUS (HPV) SEROPREVALENCE AND ANOGENITAL HPV DETECTION AMONG YOUNG HETEROSEXUAL MEN

02. Epidemiology and natural history

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Background / Objectives

HPV seroprevalence is a marker of cumulative HPV exposure, and though imperfect, can provide a simple measure of HPV exposure in a population. We studied HPV seroprevalence and anogenital detection at baseline in 3,463 heterosexual men (HM) 17-27 years old participating in a multinational clinical trial of the quadrivalent HPV vaccine.

Results

3 extra genital swab (Penile, scrotal, perineal/perianal) samples were collected at baseline and analyzed for 14 HPV types, including types targeted by the 9-valent (9v) HPV vaccine (6/11/16/18/31/33/45/52/58). Seropositivity was measured for the 9v types with a highly specific and sensitive competitive luminescence immunoassay (cLIA).

Conclusion

At baseline, 455 (13%) HM had HPV anogenital detection of at least one 9vHPV type; of these, 13% were seropositive to the same HPV type and 34% were seropositive to any 9v type. Among these 455 men, seropositivity concordant with the same HPV type was: HPV6 (34%), HPV11 (20%), HPV16 (7%), HPV18 (3%), HPV31 (4%), HPV33 (9%), HPV45 (5%), HPV52 (5%), HPV58 (11%). Concordance between anogenital detection at baseline and seropositivity to the same HPV type varied by anatomic location of the swabs. Perianal/perineal swabs had higher concordance between seropositivity and anogenital detection [16.4% (10.7-23.6), overall for any 9vHPV type] as compared to penile [13.0% (9.95-16.7), any 9vHPV

type] and scrotal swabs [11.6% (8.03-16.1), any 9vHPV type]. The older age group of HM (21-27 years old) had overall higher concordance between anogenital detection at baseline and seropositivity to same HPV type [16.4% of 21-27 y.o. vs 10.5% of 16-20 y.o. were seropositive to any 9v type]. In a sub-analysis of a random sample of 208 HM from the US with no HPV detected at baseline, 13% were seropositive to at least one 9vHPV type.

References

Young HM showed evidence of past and current exposure to 9vHPV types. These findings support early age at HPV vaccination in males to maximize vaccine preventive benefit prior to sexual debut.

00500

COMPARISON OF 2-DOSE AND 3-DOSE REGIMENS OF 9-VALENT HPV VACCINE: RESULTS FROM A 3-YEAR RANDOMIZED IMMUNOGENICITY TRIAL

05. HPV prophylactic vaccines

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Background / Objectives

We report 3-year persistence of HPV-antibody responses to the 9-valent HPV (9vHPV) vaccine among girls/boys receiving 2-dose regimens versus girls and young women receiving 3 doses.

Results

In this international, randomized immunogenicity trial (NCT01984697), girls (age 9-14 years) received 2 doses of 9vHPV vaccine (Months 0,6 [n=301] or 0,12 [n=151]) or 3 doses (Months 0,2,6 [n=301]); boys (age 9-14 years) received 2 doses (Months 0,6 [n=301] or 0,12 [n=150]); and women (age 16-26 years) received 3 doses (Months 0,2,6 [n=314]). Anti-HPV geometric mean titers (GMTs) and seropositivity rates were assessed by competitive Luminex immunoassay through Month 36.

Conclusion

Anti-HPV GMTs were highest 1 month after completing the 2-dose or 3-dose series, decreased sharply during the subsequent 6 to 12 months, then decreased more slowly through Month 36. At Months 24 and 36, GMTs in girls and boys given 2-dose regimens were generally similar to or greater than those in women given 3 doses. Month 36 seropositivity rates were ≥83.6% and 81.4% in girls and boys, respectively, vaccinated at Months 0,6; 87.9% among girls/boys vaccinated at Months 0,12; and 91.2% and 77.8% in girls and women, respectively, who received 3 doses.

References

HPV antibody responses persisted through 3 years in girls and boys who received 2 doses of 9vHPV vaccine, with GMTs similar to or greater than those observed in young women receiving 3 doses. Antibody responses generated by 2 doses in girls

and boys may be sufficient to induce high-level protective efficacy through 36 months post-vaccination onset.

00504

LONG-TERM EFFECTIVENESS AND IMMUNOGENICITY OF QUADRIVALENT HPV VACCINE IN YOUNG MEN: 10-YEAR END-OF STUDY ANALYSIS

05. HPV prophylactic vaccines

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Background / Objectives

We report the 10-year, end-of-study analysis of a long-term follow up (LTFU) study that assessed the effectiveness and immunogenicity of the quadrivalent human papillomavirus (qHPV) vaccine in men.

Results

In the 3-year base study (NCT00090285), young men (16-26 years old) were randomized 1:1 to receive 3 doses qHPV vaccine or placebo; results from participants who received 3 vaccine doses and participated in the LTFU are reported. Participants were assessed annually in the 7-year LTFU for HPV6/11-related genital warts and HPV6/11/16/18-related external genital lesions (EGL), and a subpopulation was assessed for HPV6/11/16/18-related anal intraepithelial neoplasia (AIN) or anal cancer. Persistence of anti-HPV6/11/16/18 antibodies was evaluated from serum samples collected 48-72 months (first LTFU visit) and 10 years post-Dose 1.

Conclusion

A total of 917 participants were followed for effectiveness for up to 11.5 years (median: 9.5 years) post-Dose 3. There were no new cases of HPV6/11-related genital warts, HPV6/11/16/18-related EGL, or HPV6/11/16/18-related high-grade AIN during the LTFU in the per-protocol population. One low-grade AIN (AIN1) with positive PCR results for HPV6 and HPV58 was reported. Seropositivity rates based on competitive Luminex immunoassay were >97% at Month 7; remained high over time for HPV6/11/16; and decreased for HPV18 (40.2% at Month 120). Seropositivity

rates at Month 120 assessed by IgG Luminex immunoassay (a more sensitive assay) were>90% for all 4 HPV types.

References

The qHPV vaccine provides durable protection from vaccine-type–related anogenital disease and elicits persistent HPV antibody responses through 10 years post-vaccination onset in 16-26–year-old men.

00583

EFFICACY, IMMUNOGENICITY AND SAFETY OF THE QUADRIVALENT HPV L1 VIRUS-LIKE PARTICLE (VLP) VACCINE IN 16- TO 26-YEAR-OLD JAPANESE MEN

05. HPV prophylactic vaccines

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Background / Objectives

The quadrivalent human papillomavirus L1 VLP (qHPV) vaccine protects against infection and disease related to HPV6/11/16/18. A Phase 3 efficacy, immunogenicity, and safety study of qHPV vaccine was conducted in Japanese men.

Results

In this randomized, double-blind study (NCT01862874), 16–26-year-old Japanese men received 3 doses of qHPV vaccine or placebo (Day 1, Month 2, Month 6). Serum was collected at Month 7 (i.e., 4 weeks post-Dose 3) and Month 36 for analysis of vaccine HPV type antibody responses. Swab samples were collected for analyses of persistent infection. The primary efficacy analysis was performed at an interim analysis; we report end of study results through Month 36. Efficacy and immunogenicity analyses were based on per-protocol populations that included participants who received all 3 vaccinations and were HPV-naïve prior to Day 1 through Month 7 for the relevant type. Vaccine-related serious adverse events (SAEs) were collected throughout the study.

Conclusion

A total of 1124 Japanese men were randomized, 1062 completed the 3-dose vaccination series, and 968 completed the 36-month study. Anti-HPV6/11/16/18 responses in the qHPV vaccine group were markedly induced at Month 7; >97% of participants who received qHPV vaccine seroconverted to each vaccine HPV type at Month 7, and 60.7–92.3% of qHPV vaccine recipients remained seropositive to each HPV type at Month 36. Efficacy of qHPV vaccine against HPV6/11/16/18-related 6-month persistent infection was 85.9% (95% CI: 52.7, 97.3). There were no vaccine-related SAEs or discontinuations due to an adverse event (AE) in the qHPV vaccine group; 3 placebo recipients discontinued due to AEs.

References

The qHPV vaccine was highly immunogenic and efficacious in preventing HPV6/11/16/18/-related persistent infection in Japanese men. The qHPV vaccine was generally well tolerated in this population.

00620

HPV IN MALES: RATIONALE FOR GENDER-NEUTRAL VACCINATION

05. HPV prophylactic vaccines

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Background / Objectives

The incidence of HPV-related disease is high in men and women. Globally, there are >92,000 new cases of oropharyngeal cancer, >48,000 cases of anal cancer and >34,000 cases of penile cancer annually, as well as approximately 32,000,000 cases of genital warts. Effective vaccination programmes are required to reduce the burden. HPV types 6 and 11 are responsible for >90% reported cases of genital warts and recurrent respiratory papillomatosis, and types 16 and 18 for approximately 30% to 90% of reported cases of penile, anal and HPV-associated oropharyngeal and oral cavity cancers. HPV-related cancer incidence is increasing in men. Analysis of a multinational, prospective study evaluating genital, anal and oral HPV natural history, and HPV-related external genital lesions in men (HIM study) reported a higher genital HPV prevalence in men compared with women; furthermore, this prevalence did not vary with age. Oral HPV prevalence is significantly higher in men vs women, and is shown to increase with age in men; incidence is highest at 31 to 50 years of age and lowest at 18 to 30 years of age. In addition, incidence of HPV-related infection of the anal cavity does not vary with age in men. A low rate of seroconversion (at 24 months) following HPV infection is observed in men compared with women. With the exception of HPV type 18, seropositivity is not associated with a lower incidence of HPV infection and, therefore, recurrence of genital HPV infection (high-risk HPV types [16/18/31/33/45/52/58]: 12.8% [incident]; 22.9% [prevalent]) and genital warts is high in men. The data presented herein highlight that men rarely develop immunity following natural HPV infection, regardless of age, and that antibodies acquired from natural HPV infection do not protect against subsequent HPV infection or resultant disease. Men remain susceptible to HPV infection and related diseases throughout the lifespan.

Results

N/A

Conclusion

N/A

References

Adding males to HPV vaccine programmes will result in direct protection against HPV infection, greater and faster disease reductions in females, and add resiliency to national HPV vaccination programmes.

References

N/A

00626

Long-Term Follow-Up Study of Immungenicity and Effectiveness of the 9-Valent HPV (9cHPV) Vaccine in Preadolescents and Adolecsnets (9-15 y.o.)

05. HPV prophylactic vaccines

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00508

A SYSTEMATIC LITERATURE REVIEW OF COST EFFECTIVENESS STUDIES ASSESSING THE NONAVALENT HUMAN PAPILLOMAVIRUS (HPV) VACCINE IN A GENDER NEUTRAL POPULATION

32. Economics and modelling

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Background / Objectives

The nonavalent vaccine protects against nine types of HPV (6/11/16/18/31/33/45/52/58), as opposed to four types with the quadrivalent vaccine (6/11/16/18) and two types with the bivalent vaccine (16/18). Following recommendations from national health authorities, several countries have implemented HPV gender neutral vaccination (GNV) programs. The objective of this systematic literature review was to identify and summarize all available evidence on the cost-effectiveness of national nonavalent HPV vaccination programs in a gender neutral

Results

MEDLINE, EMBASE, Cochrane CENTRAL, EconLit and NHS EED were systematically searched for cost-effectiveness analyses published in the last 10 years in English that met the pre-set eligibility criteria. The Drummond checklist was used to assess the quality of included studies.

Conclusion

Eight studies, based on four model types, were identified from five countries. The main study characteristics and results for nonavalent GNV versus quadrivalent GNV and/or girls' quadrivalent vaccination are presented in Table 1. The incremental cost-effectiveness ratio (ICER) did not exceed the respective local willingness to pay

thresholds in any of the studies reporting these comparisons (n=3). The ICERs were most sensitive to vaccine cost, discount rate and duration of protection parameters although these remained cost-effective. None of the studies included costs related to work productivity loss.

References

Across the five countries with published evidence, HPV GNV with a nonavalent vaccine was cost-effective, cost-saving or dominating compared with a gender neutral quadrivalent vaccination or girls' quadrivalent vaccination. This study supports the continued implementation of HPV vaccination with the nonavalent vaccine on a gender neutral population.

Table 1 Study characteristics and main results

Study	Country	Model type	Vaccine doses	Currency year	Time horizon	Discount rate	Herd immunity?	ICER/QALY 9v GN vs 4v GN	ICER/QALY 9v GN vs 4v girls only
Brisson 2016	United States	Individual based transmission dynamic model†	3	US$ 2010	70 years	3%	Yes	Cost-saving	NR
Simms 2016	Australia	Individual based transmission dynamic model†	2	AUS$ 2013	NR	5%	Yes	NR	Cost-effective, maximal additional cost per dose (9v over 4v): 22.74
Mennini 2017	Italy	Deterministic, dynamic, population based model‡	2	€ 2014	100 years	3%	NR	10,463	13,541
Largeron 2017	Germany	Deterministic, dynamic, population based model‡	2	€ 2014	100 years	3%	No	NR	22,987
Laprise 2016	United States	Individual based transmission dynamic model	2 or 3	US$ 2013	100 years	3%	NR	NR*	NR*
Boiron 2016	United States	Deterministic, dynamic, population based model‡	3	US$ 2015	100 years	3%	NR	16,441	NR
Duhram 2016	Austria	Age structured compartmental model‡	2	€ 2014	2015 to 2050	3%	Yes	Dominates (vs. 2v/4v)	NR
Chesson 2016	United States	Deterministic, dynamic, population based model	3	US$ 2013	100 years	3%	Yes	8,600	NR

2v: bivalent HPV vaccine; 4v: quadrivalent HPV vaccine; 9v: nonavalent HPV vaccine; GNV: gender-neutral vaccination; ICER: incremental cost-effectiveness ratio; NR: not reported; QALY: quality adjusted life-year

† Based on HPV-ADVISE, http://www.marc-brisson.net/HPVadvise-US.pdf

‡ Based on Elbasha EH, Dasbach EJ. Impact of vaccinating boys and men against HPV in the United States. Vaccine. 2010;28(42):6858–6867

* Laprise 2016 compared a 2-dose nonavalent vaccination schedule with no vaccination, and a 3-dose nonavalent vaccination schedule with a 2-dose vaccination schedule. A 2-dose vaccination schedule was shown to be cost-saving compared to no vaccination. A 2-dose vaccination schedule that provides at least 20 years of protection is cost-effective compared to a 3-dose vaccination schedule. However, if a 2-dose vaccination schedule provides less than 20 years of protection while a 3-dose vaccination schedule provides more than 20 years of protection, the 3-dose vaccination schedule yields a substantial increase in QALYs gained.

00501

GENDER-NEUTRAL HPV VACCINATION: FACILITATORS AND BARRIERS TO EXPANDING COVERAGE TO MALES

36. Public health

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Background / Objectives

A greater understanding of the impact of Human Papillomavirus (HPV)–related diseases on males and females has prompted 18 countries to expand their HPV vaccination programs to include both genders. However, broad international support for gender-neutral HPV vaccination (GNV) programs has not been realized. This study aims to capture the experiences and challenges of countries that have implemented GNV programs to share best practices with countries considering similar expansions.

Results

A qualitative study involving in-depth interviews of experts (n= ~18-24) involved in HPV GNV programs in 6 countries (Argentina, Australia, Austria, Brazil, Canada and Italy) is currently underway. The countries were selected based on a literature review of countries with existing GNV programs. Using a semi-structured interview guide, we have obtained insights into countries' decision-making processes for GNV expansion, key strategies and challenges encountered during program implementation, and recommendations for other countries considering expansion. Interviews were audio-recorded and transcribed verbatim in English and/or the local language.

Conclusion

Using a qualitative approach, data from interviews conducted to date were reviewed and coded to identify key themes. Emergent themes encompass factors associated with the selected countries' decision to expand and implement GNV programs. These include the government or health ministry's prioritization of HPV vaccination relative

to other vaccination programs, its role in maintaining and disseminating up-to-date vaccination and disease-related information once a GNV program has been implemented and the role of key stakeholders including media, advocacy groups, and policymakers in supporting GNV programs. To overcome challenges related to these themes, participants made the following recommendations: (1) continuous educational programs for clinicians, school-based providers, and the general population to increase awareness of the HPV-related disease burden, efficacy of the vaccine, and its use in males; (2) utilization of positive outreach to disseminate the benefits of the vaccine to target populations; and (3) implementation of school-based, rather than clinic-based, vaccination programs to increase vaccine uptake.

References

Our initial analysis reveals various factors should be considered when implementing a GNV program, including the education of healthcare providers and support from various stakeholders, including government entities. Moving forward, countries will need to address the gaps between the science and the public's knowledge to effectively and efficiently maintain GNV programs.

FC 05. Epidemiology

00047

AGE-SPECIFIC CERVICAL CANCER INCIDENCE AFTER ELIMINATION OF DIFFERENT VACCINE-PROTECTED HPV TYPES

02. Epidemiology and natural history

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Background / Objectives

By high efficacy of HPV vaccines, several mathematical modelling studies suggest that the elimination of vaccine-protected HPV types is an achievable goal. As different HPV types are associated with invasive cervical cancer (ICC) to a different extent in different age groups, large and comprehensive HPV genotyping series are required for accurate data-driven predictions.

Results

We used the HPV genotyping data (N=2950) that were obtained from the archival tumor blocks of all reported ICC cases in Sweden during a 10-year period (N=4253) in a national study performed by the Swedish National Cervical Screening Registry. We estimated the baseline and the remaining age-specific HPV-related ICC incidences, after eliminating different groups of vaccine-protected HPV types from the population, and the corresponding standardized lifetime risks (SLTR) per 100,000 female births.

Conclusion

The baseline SLTR of HPV-positive ICC was 650 cases per 100,000 female births. After eliminating vaccine types HPV16/18 the SLTR was reduced to 157 for the remaining HPV-positive ICC (24% of HPV-positive SLTR remaining). For the vaccine-targeted and cross-protected types of bivalent, quadrivalent, and nonavalent vaccines, the corresponding remaining SLTRs were 69 (11%), 130 (20%), and 47 (7%), respectively.

References

The predicted population level ICC incidences demonstrate the preventive potential of optimal high-coverage vaccination programs, beyond the vaccine efficacies. The incidences after eliminating all vaccine-protected HPV types are very low, predicting substantial health benefits of effective vaccination programs.

00140

DISTINCT INCREASE IN CERVICAL PRECANCERS IN NORWAY IS EXPLAINED BY BOTH INCREASED EXPOSURE TO HPV AND IMPROVED SCREENING METHODS: NATIONWIDE STUDY FROM 1992 TO 2016

02. Epidemiology and natural history

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Background / Objectives

The aim of this study was to examine the incidence of cervical intraepithelial neoplasia grades 2 and 3 (CIN2, CIN3) and adenocarcinoma in situ (AIS) in Norway during the period from 1992 to 2016 in detail.

Results

From the Cancer Registry of Norway, we identified all incident cases of CIN2, CIN3, AIS and cervical cancers. We used the triannual percentage change statistic for describing alteration in age-specific incidence rates (IR) and age-standardised IRs. The age-period-cohort model was used to distinguish between period and cohort effects. Changes in the coverage of nation-wide screening program and changes in the screening technology used were assessed.

Conclusion

In 2014-2016, women aged 25-29 years of age carried the highest burden of cervical precancers, with the IR of $737/10^5$ for CIN3, $193/10^5$ for CIN2, and $32/10^5$ for AIS. The IR of CIN2 and AIS increased for all age-groups with 20% (95% CI: 2; 43) and with 24% (95% CI: 13; 36) at every 3-years period. From 2011 to 2016, overall 41% increase of CIN3 was observed, whereas the increase was most profound among 25-29-year-old women (62%, 95% CI: 29; 102). Cancer incidence was stable or decreasing. Since 2006, the proportion of screening performed with liquid-based

cytology (LBC) started to increase, escalating in 2010, and in 2016, 86% of all the screening tests performed in Norway were LBC.

References

Some of the observed cohort effects can be attributed to the increased background risks for HPV infection. Age at first sexual intercourse has decreased, while the number of lifetime sexual partners has increased leading to higher exposure to sexually transmitted infections, including HPV. However, a period effect caused by changes in screening and histological verification practices of precancerous lesions may also play the role of the steady increase in incidence over the 25-year study period. For effective control of cervical cancer, treatment of screening-detected cervical precancerous lesions is needed. The observed increase in cervical precancers presents a challenge to assess the effect of cancer prevention programs. Nation-wide registries recording detailed information on screening technology, outcomes and HPV vaccination status will be useful in developing age-appropriate cancer prevention program with minimal overtreatment and related health risks.

00151

HPV type replacement: still too early to tell?

02. Epidemiology and natural history

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Background / Objectives

HPV vaccination has proven effective in reducing transmission and prevalence of the vaccine-targeted and cross-protective types. However, concerns have been raised whether other non-vaccine types (NVT's) will fill the vacated ecological niche. So far, most observational surveillance studies found no evidence for type replacement based on 1) a lower incidence of NVT's among vaccinated compared to unvaccinated individuals in randomized controlled trials; and 2) no significant increase in the prevalence of NVT's within ten years of follow-up compared to the pre-vaccination period. We investigate whether these observations are conclusive for excluding type replacement in the long run.

Results

We studied how the prevalence of a NVT evolves after the introduction of vaccination using an age-structured transmission model with two competing types. We investigated how the timing and strength of type replacement depend on the strength and mechanism of competition, cross-protection and vaccination coverage.

Conclusion

We found scenarios compatible with observations 1) and 2) but yielding type replacement in the long run. Cross-protection may lead to observation 1), although type replacement could occur throughout the population. We identified low coverages under the threshold coverage and competition later in the infection episode to slow down type replacement, so that a follow-up of ten years may fall short. When cross-protection is present but too weak to prevent type replacement, the prevalence of the NVT may even decrease initially before increasing ultimately.

References

Although present evidence is reassuring, trend analyses with longer follow-up are required to rule out type replacement.

00152

EFFECT OF CHANGES TO THE AGE AT FIRST INVITATION TO SCREENING ON MORTALITY FROM CERVICAL CANCER IN ENGLAND

02. Epidemiology and natural history

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Background / Objectives

In 2004 the age of first cervical screening invitation in England was increased from 20 to 25. (1) This reflected evidence that screening at ages 20-24 provided no population benefit in terms of cancer prevention.(2) In 2012, the age of sending out the first screening invitation was changed again; this time to 24.5 years. To enable women to be screened by their 25th birthday.(3)

We previously reported (4) that inviting women at age 25.0yrs was associated with an increase of 43.7 cancers per 100,000 women-years (95%CI: 37.4 to 49.9, $p<0.001$). The increase in the number of cancers diagnosed at age 25 was restricted to cancers stage IA or IB; no increase in advanced (II+) was observed.

Here we aim to explore the effect the age at first screening invitation has had on mortality from cervical cancer.

Results

We have requested data from the national cancer registry in England on number of diagnosis of cervical cancer (ICD10- C53) and estimates by stage of survival at 1, 2, 3, 4 and 5 years after cancer diagnosis.

We have requested that women are grouped (based on year of birth and age at diagnosis) as follows:

1. Aged 20.0-24.5 at diagnosis and diagnosed from 2006 onwards

2. Age 26.0-29.99 at diagnosis and diagnosed 2006 onwards, excluding diagnoses in the first six months of 2009 and the last 4 months of 2008. Also excluding any aged 28 in 2012 onwards.

3. Age 25.0-25.5 at diagnosis in 2009-2016, aged 24.5-25.0 at diagnosis in 2013-2016, Age 25.5-26.0 at diagnosis in 2010-2015.

4. Age 25.0-25.5 at diagnosis in 2006-2008, aged 24.5-25.0 at diagnosis in 2006-2012, Age 25.5-26.0 at diagnosis in 2006-2009.

5. Age 28 at diagnosis in 2012-2016.

Conclusion

We will report KM survival estimates by stage and age at first invitation for screening (as per the groups above). We hypothesis that groups 1, 2 and 4 will all have similar survival (hazard of about 0.9% per year); that the hazard in group 3 would be the lowest (about 0.25% per year) and the hazard in group 5 would be in between (about 0.45% per year). We should have sufficient power to show lower fatality in group 3 compared to group 2.

The following limitations should be considered. We will have no information on when or whether women were invited for screening prior to diagnosis (since these data are not linked to screening data). Not all women will have 5-year follow-up. The analysis is subject to lead-time bias; however, our main interest is to assess whether what we are seeing cancers with lots of lead time or cancers that should have been prevented.

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Pending

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00174

EPIDEMIOLOGY AND CONTROL OF CERVICAL CANCER IN BRAZIL – ROLE OF HPV GENOTYPES

02. Epidemiology and natural history

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Background / Objectives

The estimated age-adjusted incidence of cervical cancer (CC) in Brazil is approximately of 18/100,000 women/year, with large regional differences. Apart from epidemiological interest, recognizing the role of each individual HPV genotype in cervical malignancy became more important after the introduction of HPV vaccines against HPV 16 or 18 infections, showing limited cross protection to other high-risk genotypes non-16/18. Not surprisingly, a new generation of vaccines including another 5 high-risk HPV genotypes have recently been launched. Surveillance with extended genotyping platforms is necessary to identify an eventual emergence of a non-vaccine genotype that may occupy this, to be vacant, ecological niche. However, it is clear that the current vaccines are highly effective on naïve populations but not appropriate to previously and current infected subjects. This translates into the need to screen for CC for the next 50 years. Concerning CC screening, several studies have shown that testing for HPV-DNA is more advantageous than by cytology. Therefore, many countries are remodeling their CC screening program, placing HPV testing as the primary tool and referring to cytology only the HPV+ samples. The objective of this work is to investigate the differential role of HPV genotypes in the epidemiology of cervical cancer in Brazil.

Results

Brazilian studies investigating the distribution of HPV genotypes in the general population and cervical cancer specimens were reviewed and compiled.

Conclusion

Prevalence of high-risk HPVs in Brazil is similar to countries presenting a low incidence of CC, indicating inefficiencies in the national screening program, which relies on cytology. HPV genotyping in large scale has depicted a frequency of HPV genotypes in the general Brazilian female population that is similar to the global

distribution; HPV 16 being the commonest followed by other genotypes according to the study population and geographical region, but not HPV 18. In contrast, on CC cases HPV 18 is the second, being present on 10-15% of all CCs, far after HPV 16 which accounts for 50-70%.

References

Large studies with prolonged follow-up revealed that among HPV genotypes, classified as of high-risk, there are significant differences in their oncogenic potential. Consequently, in the age of personalized medicine, it makes sense to have management strategies according to the genotype or group identified in the sample. Several biomarkers are under evaluation in order to identify on HPV positive cervicovaginal samples, those that present a higher diagnostic or prognostic risk of neoplasia, thus justifying more costly and invasive procedures.

00226

ESTIMATING INCIDENCE RATES OF GROUPED HPV TYPES: A SYSTEMATIC REVIEW AND ANALYSES OF THE IMPACT OF DIFFERENT EPIDEMIOLOGICAL ASSUMPTIONS

02. Epidemiology and natural history

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Background / Objectives

The incidence rate (IR) is an important metric for the occurrence of new cases in a population at risk and is commonly used in studies on HPV. Some studies provide not only type-specific IRs, but also IRs of grouped HPV types, e.g. the IR of all high-risk (HR) HPV types. Researchers take different approaches to calculate such IRs, which may mean that estimates of IRs between studies are not comparable. Here, we assessed the impact of different epidemiological assumptions on the estimated IRs of grouped HPV types.

Results

We first performed a systematic review exploring the approaches used to estimate IRs; papers published from 2010 onwards were included. Subsequently we applied these approaches to data of the H2M study, an observational cohort study of HPV in men who have sex with men. IRs were estimated for six HPV groupings: any HPV, HR-HPV, low-risk (LR) HPV, 2vHPV, 4vHPV, and 9vHPV. We used the midpoint assumption for all analyses, meaning that we assumed an incident event occurred at the midpoint between the last negative and first positive HPV test result.

Conclusion

The systematic review yielded six different approaches (A to F). IRs according to A, B and C, excluded participants who were positive for any of the HPV types of a grouping at baseline, while for approaches D, E and F IRs were estimated regardless of baseline HPV status. Approaches A and D took only the first incident event into account, while approaches B, C, E and F also took subsequent incident events, at later visits, into account. Approaches B and E considered multiple incident events

detected at the same visit as one incident event, while approaches C and F considered each incident event, regardless of timing.

Applying these six approaches to the H2M data (n=749, median follow-up time: 24.2 months), we found large differences in the number of included participants at baseline, the number of incidents events, person-time and the IR. For example, for the HR-HPV group, 356 participants were at risk according to approaches A, B and C, and 749 according to approaches D, E and F; the number of incident events varied between 215 and 975; person-months varied between 5,115 and 17,602; and the IR varied between 3.50 and 5.59 per 100 person-months. The estimated IRs, per HPV grouping, for the six approaches are shown in the figure.

References

In published studies different epidemiological assumptions are used to estimate IRs of grouped HPV types, leading to six different approaches; these approaches lead to widely differing estimates of IRs. Meaning that the interpretation of grouped HPV IRs is not straightforward and that comparison of these IRs between studies is not warranted.

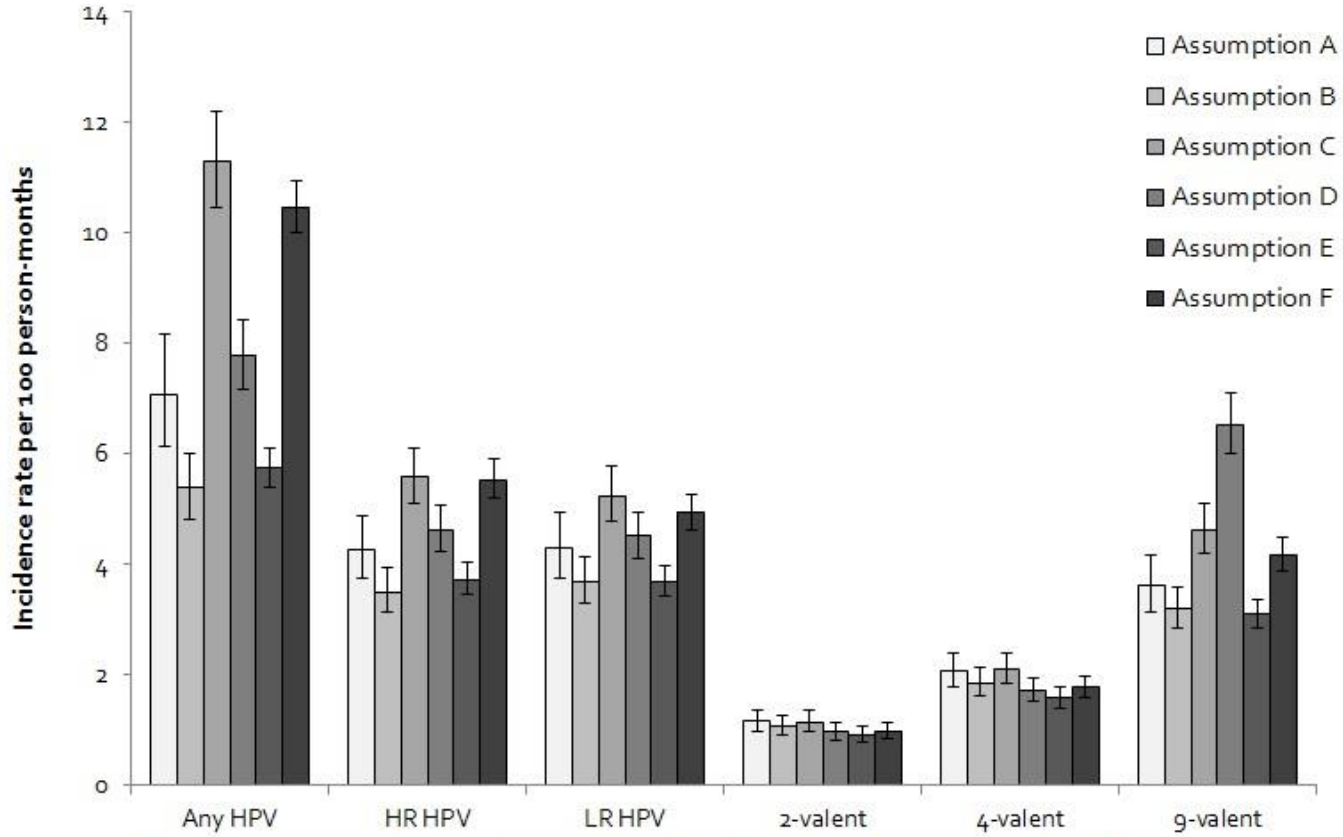

Figure: Incidence rates per 100 person-months of six approaches, shown for six HPV groupings

00410

Prevalence of Vaccine-Targeted High-Risk HPV Types Among Mid-Adult Women in Europe

02. Epidemiology and natural history

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Background / Objectives

Cervical HPV prevalence in the general female population varies by age. We compiled published information on the prevalence of vaccine-targeted high-risk (HR) HPV types in mid-adult women (age 25–45 years) in Europe.

Results

PubMed/EMBASE were systematically searched for original studies, published from January 2013–October 2017, reporting type-specific cervical HPV prevalence among the general population of mid-adult women in Europe. Studies reporting HPV DNA detection of types 16, 18 and ≥1 of 31, 33, 45, 52, or 58 in populations screened for cervical cancer with a mean or median age between 30 and 40 years were included. Key exclusion criteria were age <25 or >45 years only, small study (N<300), not English, and diseased or high-risk population. Data extracted included, type of population, age information, HPV typing and sample collection methodology. Data were pooled on a country- and sub-region level.

Conclusion

Twenty-seven publications were identified: 4 for Eastern Europe (Bulgaria, Czech Republic, Romania; N=4,414 women), 7 for Northern Europe (Denmark, Norway, United Kingdom; N=75,369), 9 for Southern Europe (Croatia, Greece, Italy, Portugal, Serbia, Slovenia, Spain; N=26,136) and 7 for Western Europe (Austria, Belgium, France, Germany; N=8,890). Where reported, vaccination rates were ≤6%. Reported prevalences varied on study and sub-region levels. The pooled prevalence of cervical HPV 16 was 10.8% (range 4.8–17.9% across 4 studies) in Eastern Europe, 5.1% (3.0–6.8%, 7 studies) in Northern Europe, 7.3% (3.7–18.9%, 9 studies) in Southern Europe, and 4.2% (2.1–7.6%, 7 studies) in Western Europe. Pooled prevalence of

HPV 18 was 3.0% (1.8-3.9%, 4 studies) in Eastern, 2.2% (1.1–2.9%, 6 studies) in Northern, 2.1% (1.0–7.8%, 9 studies) in Southern, and 1.0% in Western Europe (0.5–2.1%, 7 studies); prevalence of HPV 31 was 3.5% (1.2–5.0, 4 studies) in Eastern, 3.6% (1.8–4.7%, 5 studies) in Northern, 3.9% (2.0–16.0%, 8 studies) in Southern, and 2.2% (1.2–4.1%, 6 studies) in Western Europe; and prevalence of HPV 52 was 2.7% (1.3–4.0%, 4 studies) in Eastern, 3.8% (1.4–4.9%, 5 studies) in Northern, 2.3% (0.9–6.3%, 8 studies) in Southern, and 1.6% (1.1–3.8%, 6 studies) in Western Europe. Prevalences of HPV types 33, 45 and 58 ranged within 0.9% to 2.5% across sub-regions. Across 3 studies stratifying data by 5-year age sub-groups, HPV prevalences generally decreased with increasing age within the 25–45-year age range.

References

Although estimates vary, HPV 16 is the most common vaccine-targeted HR type in mid-adult European women, followed by 31 and 52 in all regions except Eastern Europe, where type 18 is the third most common type after 31.

00440

Cervical cancer incidence and mortality trends in Latvia in 1993-2016

02. Epidemiology and natural history

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Background / Objectives

Cervical cancer incidence and mortality in Latvia remains among highest in Europe[1,2]. Nationwide organised cervical cancer screening programme was introduced in Latvia in 2009. However, participation rates are unsatisfactory low, below 40% in 2017 [4]. The aim of this study was to examine recent trends in incidence and mortality rates for cervical cancer from 1993 to 2016 by age groups and by the place of residence, and to evaluate the potential impact of the screening program.

Results

The study includes data from the population-based Latvian cancer registry introduced in 1993. The sample includes female patients with diagnosed and histologically confirmed cervical cancer from 1993 to 2016. Data from the Death causes database of Latvia was used for development of the cancer-specific mortality estimates. Age-standardized incidence and mortality rates were calculated by direct standardization method using world standard population. Incidence and mortality changes were detected with joint point regression method using the National Cancer Institute program Joinpoint Software 4.4.0.0 [4].

Conclusion

Age-standardized cervical cancer incidence in Latvia increased from 9.7 to 15.8 per 100 000 females by 2.9% (95% CI: 2.3-3.4%) annually and standardized mortality rose from 4.4 to 5.7 per 100 000 females on average by 1.8% (95% CI: 0.9 -2.8%) annually without any significant changes in trend lines. Most rapid incidence increase was in the age groups 25-39: on average by 4.1% (95% CI: 3.2-5.1) and 40-54: 4.0% annually (95% CI: 3.2-5.1). The greatest increase in cancer-specific mortality was

observed in the age group 40-54: 2.8% average annually (95% CI: 1.4-5.2). The highest incidence rates with the most rapid increase was observed among women living in small towns or rural area: average annual increase by 3.0% (95% CI: 2.3-3.6).

References

Incidence and mortality trends of cervical cancer in Latvia do not show any changes since introduction of the organised population-based screening programme in 2009 and reflects low programme coverage. In developing further activities to improve the coverage women from 25 to 54, as well as those living in small towns and rural area.

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00474

LONG-TERM CERVICAL CANCER RISK FOLLOWING HPV INFECTION – 28 YEAR FOLLOW-UP OF THE MANCHESTER COHORT

02. Epidemiology and natural history

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Background / Objectives

The natural history of HPV infection and subsequent invasive cancer development can only be studied over long periods and in large cohorts. Decisions on implementing primary HPV testing are often based on studies with CIN2 or CIN3 as the primary outcome.

Results

Between 1988 and 1993, in collaboration with over 100 general practitioners and screening clinics in the Greater Manchester area, cervical cell samples were collected from 49,655 women attending for routine cytology screening. There was no age restriction. HPV testing was carried out between 1990 and 1996 on a random sample of 7278 of the women in the cohort, 6462 of whom gave a satisfactory β-globin result. PCR analysis was by HPV L1 MY09/MY11 consensus primers. We have followed the trial cohort to October 2016 through national cancer registration for CIN3 and cancer, giving a median follow-up of 26 years.

Conclusion

47,625 women (96%) were successfully traced. Follow-up identified 1143 cases of CIN3 and 138 invasive cervical cancers. Stored samples from cervical cancers, CIN3s and random controls are being tested for HPV. A preliminary analysis included 126 cases of CIN3 and 17 invasive cervical cancers among 6215 women whose entry sample was tested for HPV in 1990-1996. The cumulative invasive cervical cancer risk 28 years after testing positive for HPV 16/18/39/45 (296 women) was 3.4% (95% CI: 1.8%-6.7%: 9 cancers) and for other HR-types (143 women) was 0.7% (95% CI: 0.1%-5.0%: 1 cancer). The cumulative risk following a negative HPV test (5,776 women) was 0.13% (95% CI: 0.06%-0.28%: 7 cancers). Table 1 shows a

decreasing ratio of CIN3 to invasive cancer with increasing time. CIN3 was rarely diagnosed above age 45. This pattern was also seen among the 439 women testing HRHPV positive.

Table 1: CIN3 and cervical cancer registrations by time since Manchester Study (1988-93) in 49,625 women (upper part) and 6,215 women tested for HPV (lower part)

Time since study	**<5 years**	**5-9.9 years**	**10-14.9 years**	**15-19.9 years**	**20-24.9 years**	**≥25 years**	**Total**
All women (n=47,625)							
CIN3	345	403	256	92	43	4	1143
Cervical cancer	32	28	30	23	21	4	138
CIN3:cancer ratio	10.8	14.4	8.5	4.0	2.0	1.0	
6,215 women HPV tested at entry							
HPV+ (n=439)							
CIN3	32	10	7	0	0	0	49
Cervical cancer	3	3	1	1	1	1	10
HPV- (n=5,776)							
CIN3	21	31	14	8	3	0	77
Cervical cancer	1	1	2	1	2	0	7

References

CIN3 risk declined sharply beyond 5 years after HPV detection, however the invasive cancer risk remains elevated into middle and old age. More detailed results by age and HPV status will be presented.

00054

Socioeconomic factors associated with HPV testing in the National Cancer Data Base

08. HPV testing

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Background / Objectives

HPV-associated oropharyngeal cancers have risen over the last decade to overtake cervical cancer as the most common cancer caused by HPV. Given the increased survival of HPV-associated oropharyngeal cancer and the recent staging changes of HPV-positive oropharyngeal cancer, establishing a baseline rate of HPV testing and determining factors related to HPV testing are exceedingly pertinent.

Results

We used the National Cancer Data Base, accounting for 70% of new cancer diagnoses. About 1500 Commission on Cancer accredited cancer registries submit data to NCDB using standardized data, and coding definitions. To establish a baseline and to reduce heterogeneity in early HPV-testing, we used squamous cell oropharyngeal cancer cases from 2013 to 2015. NCDB requires HPV status reported in the Collaborative Stage Site-Specific Factor 10 for all oropharyngeal cancers. Cases that have a reported HPV status, or have a test ordered but no results were classified as “tested.” If a case is missing HPV status or the record was classified as “test not done,” then cases are classified as “untested.” We used Chi-square test to compare the factors among tested and untested cases. We also calculated odds ratio comparing factors for tested or untested cases with logistic regression.

Conclusion

Overall, 11,195 cases of oropharyngeal cancer fit our inclusion criteria. Of these cases, 65.6% of cases were tested for HPV. Cases with low socioeconomic status are less likely to be tested. About 10% of the non-tested cases are African Americans, while only 7.2% of the tested cases are African Americans. Similarly, 55% of tested cases have private insurance compared to only 41% of untested cases have private insurance. When adjusted in a logistic regression, compared to cases

with private insurance, cases with Medicaid (OR: 0.56; 95% CI: 0.52, 0.61) and uninsured (OR: 0.60; 95% CI: 0.53, 0.67) are less likely to be tested. Cases who live in zip codes with high levels of high school education have 1.42 times the odds (95% CI: 1.30, 1.54) of being tested compared to zip codes in with low. There are also dramatic differences by hospital locations. Hospitals in states located in the West South Central – AR, LA, OK, TX – are far less likely (OR: 0.52; 0.45, 0.60) to test for HPV than the states in New England.

References

The results from this study suggest that cases with low socioeconomic status are less likely to be HPV tested for HPV in NCDB. This heterogeneity in testing is significant given potential de-intensification of treatment for HPV-associated cancer and to maintain equitable treatment for all.

00484

DIFFERENCES IN HIGH-RISK HPV PROFILE ACCORDING TO SEX: RESULTS OF POP-BRAZYL STUDY

09. HPV screening

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Background / Objectives

HPV is a world spread sexually transmitted infection, affecting both genders and implicated in development of different types of cancers as cervical, anal, penile and oropharyngeal. However, the infection rate and types are not equally distributed between genders. Therefore, we aim to describe the high-risk HPV profile according to sex.

Results

We analyzed data from POP-Brazil Study, a cross-sectional nationwide survey who included women and men aged 16 to 25 years. All participants answered an interview with sociodemographic and behavioral questions and provided biological samples for genital HPV analysis after signed an informed consent form. Participants were recruited in primary care unit areas by trained health professionals. We used an automated DNA extraction method (MagNA Pure LC 2.0, Roche Molecular Systems) and HPV genotyping was performed on all specimens using the Roche PCR-based Linear Array Genotyping Test (LA). In all analyses, the data were weighted by population in each capital according to sex within age range of the study. Statistical analysis was performed using SAS software (Statistical Analysis System, SAS Institute Inc., Cary, N.C.), version 9.4, and statistical significance was defined as $p < 0.05$.

Conclusion

We included 5,268 women and 1,119 men with valid samples. The mean age was 21.68 (CI95%, 21.56-21.80) years. The majority of participants self-declared as color brown 56.96% (CI95% 54.77-59.15) and pertained to social class C (55.48%, CI95% 53.32-57.64). The prevalence of high-risk HPV was 35.18% (CI95% 33.13-37.23) with significant differences between genders (38.62% in women and 29.18% in men, p = 0.0002). The high-risk profile was also different, being the most prevalent types 16 (8.92%), 52 (8.84%) and 58 (6.14%) in women, and 59 (6.43%), 51 (5.99%) and 52 (5.96%) in men.

References

Differences in high-risk HPV profile are important and can determine the incidences of HPV driven cancers. These differences must be taken into account in the prescription of HPV vaccines and primary prevention of cancer.

00078

IS EARLY AGE AT THE START OF ORAL CONTRACEPTIVE USE A RISK FACTOR OF CERVICAL ATYPIA?

22. Cervical neoplasia

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Background / Objectives

We investigated whether the risk of cervical atypia (squamous intraepithelial lesions and/or cervical intraepithelial neoplasia) is associated with a short interval between menarche and age at the first sexual intercourse (FSI), and/or between menarche and age at the start of oral contraceptive (OC) use.

Results

A total of 4808 (16-17 years old) Finnish women were enrolled in the PATRICIA trial and received either bivalent human papillomavirus (HPV) 16/18 vaccine or Hepatitis A-virus (HAV) vaccine in 2004-2005. In this study, the association of cervical atypia and interval between menarche and the FSI or age at the start OC use was assessed in the control group (2399) who received HAV vaccination and participated clinical follow-up visits every six months for four years. Altogether 914 women answered the behavioural questionnaire and had a normal baseline cervical cytology test, thereafter performed at semi-annual visits throughout the four-year trial.

Conclusion

The mean age of menarche, FSI and age at first use of OC were 12.4, 16.0 and 16.4 respectively. Among women, who had a shorter than 3 years interval between menarche and the FSI, a very high risk of cervical atypia was associated with concomitant early start of OC use (odds ratio [OR] 5.2; 95% confidence interval [CI], 3.0-9.2). When the start of use of OC was postponed beyond 3 years post menarche it was protective (OR 0.3; 95% CI, 0.2-0.4) although the interval between menarche and FSI remained less than 3 years.

References

Short interval between menarche and age at the start of OC use is a significant risk factor of cervical atypia.

00357

AGE-SPECIFIC HPV GENOTYPE DISTRIBUTION ACCORDING TO CERVICAL HISTOPATHOLOGICAL FINDINGS IN A SCREENED AND UNVACCINATED POPULATION

22. Cervical neoplasia

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Background / Objectives

HPV16 is globally the most common high-risk HPV (hrHPV) genotype and approximately 70% of invasive cervical cancers are associated with HPV16 or 18. Three prophylactic vaccines targeting the highest risk genotypes are commercially available and are in many countries implemented in the national vaccination programmes. Our objective was to study the current age-specific hrHPV genotype distribution in histopathological high-grade cervical lesions in an unvaccinated highly screened population.

Results

The HELICOPTER study (ISRCTN10933736) recruited 1383 women ≥18 years (range 19.2-83.7) attending the single referral colposcopy center in the greater Helsinki area in Finland between 2014 and 2016. Genital samples from 1360 women were tested for 36 HPV genotypes with Luminex assay. Women were divided into three age groups: <30 (n=366), 30-44.9 (n=657), and ≥45 (n=337). Referral reasons and baseline cervical histological findings were recorded, and HPV genotyping results were grouped to mimic the high-risk genotypes covered by the available vaccines: HPV16/18+ (bivalent vaccine), HPV16/18/31/33/35/45+ (bivalent vaccine with cross-protection) and HPV16/18/31/33/45/52/58+ (nonvalent vaccine). The latter two were also analysed excluding HPV16/18. The main outcome measure, histopathological high grade squamous intraepithelial neoplasia or worse (HSIL+) included HSIL, squamous cell carcinoma, adenocarcinoma in situ, and adenocarcinoma.

Conclusion

In total 295 of the 523 cases of HSIL+ (56.4%) were associated with HPV16/18: 64.2% (104/162) in women <30 years, 58.0% (164/283) in women 30-44.9 years, and only 34.6% (27/77) in women ≥45 years of age (p-test for trend 0.002). The bivalent vaccine cross-protection genotypes HPV31/33/35/45 (excluding HPV16/18) on the other hand covered 22.2% (36/162) of HSIL+ in women <30, 23.7% (67/283) in women aged 30-44.9 and increased to 26.9% (21/78) in women ≥45. Correspondingly, in the nonavalent vaccine group HPV31/33/45/52/58 (excluding HPV16/18) accounted for 26.5% (43/162) in women <30 years, 31.1% (88/283) in women 30-44.9 years, and up to 38.5% (30/78) of HSIL+ in women ≥45 years of age. The proportion of HSIL+ associated hrHPV genotypes not included in the vaccines (HPV39/51/56/59/68) also increased with advancing age, from 1.9% (3/162) in <30 years to 7.7% (6/77) in women ≥45 years old.

References

HPV genotype distribution in HSIL+ lesions is distinctly polarised, with HPV16/18 attributed disease markedly more prevalent in young women <30 than among women ≥45 years. This could have implications for the effectiveness of current prophylactic vaccines as well as for current and future screening strategies.

00142

Impact of changes in sexual behavior on past and future trends of HPV infections and related cancers

36. Public health

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Background / Objectives

Changes in sexual behavior are hypothesised to be the main cause of the substantial rise in anal and oropharyngeal cancers in the past two decades in developed countries. Linear extrapolation of current trends have shown that incidence of HPV-positive oropharyngeal cancer could exceed the number of cervical cancers by 2020. However, these projections are not informed by the possible causes of past increases, such as changes in sexual behavior over time. The aim of this study is to understand the potential impact of changes in sexual behavior on past trends of HPV transmission and to predict future trends of HPV-related cancers.

Results

We developed an individual-based model of HPV-transmission using U.S. sexual behavior data from the National Survey of Family Growth, National Health and Social Life Survey, and General Social Survey. These data cover birth cohorts from 1900 to 1999. The model population reproduces the sexual history of annual birth cohorts from 1900 to 1999. We determined, for every modelled individual, the dates of every sexual partnership and with whom it occurred. We performed simulations of HPV-16 transmission, and progression to cancer from 1900 to 2015, taking into account changes in sexual behaviour. We assumed that the probability of transmission per partnership is 100%, the average duration of infection is 1.5 years. We estimated, through model calibration, that the average duration of progression from HPV-16 infection to associated oropharyngeal cancer among men was 43 years. Finally, we assumed that the prevalence of any cofactor of HPV-16 related oropharyngeal cancer (e.g., smoking) has remained stable.

Conclusion

Table 1: Model simulations of HPV-16 prevalence by year of birth

Median prevalence of HPV-16(%)					
	Birth year				
	1900-19	1920-39	1940-59	1960-79	1980-90
Men (15-25 yrs)	3.0	6.6	11.0	13.5	14.6
Women (15-25 yrs)	0.8	2.1	5.6	9.8	11.8

Table 1: Model simulations of HPV-16 related oropharyngeal cancer by year of birth

Median incidence of HPV-16 related oropharyngeal cancer (per 100,000 person-yrs)					
	Birth year				
	1900-19	1920-39	1940-59	1960-79	1980-90
Men					
20-39 yrs	0.0	0.1	0.1	0.2	0.2
40-49 yrs	0.6	1.6	2.7	3.1	3.6
50-65 yrs	2.9	6.7	11.5	13.3	14.1

References

Our results suggest that sexual activity has stabilized for cohorts born after 1960. As a result, the increase in cancer incidence due to sexual activity is predicted to stabilize from 2025 onwards for those younger than 65 years old. Trends in sexual behavior should thus be accounted for when extrapolating HPV-related cancers, or estimating the impact of interventions.

00615

ASSESSING THE RISK OF HUMAN PAPILLOMAVIRUS TRANSMISSION AND HIGH-LEVEL DISINFECTION USING MOLECULAR VIROLOGY APPROACHES

36. Public health

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Background / Objectives

Recent studies have suggested that HPVs are not susceptible to certain high-level disinfection protocols and that medical instruments may provide transmission of nosocomial HPVs infections (1-4). We aimed to determine the infectious load of HPVs from clinical lesions and to investigate HPV virions derived from model systems and clinical lesions in their abilities to be neutralized in classical disinfection protocols.

Results

Infectious HPV virions were isolated from the 293T transfection system, organotypic epithelial tissue cultures, mouse xenografts. Clinical samples from respiratory papillomas and anogenital warts were obtained under IRB approval using emery paper to swab the apical tumors and were typed using the Seegene Anyplex™ HPV28 detection platform. A $TCID_{50}$ assay was validated using RT-qPCR approaches to measure the end-point detection of viral E1^E4 mRNAs in infected HaCaT keratinocytes. A novel focus-forming assay was validated to detect HPV E6/E7 mRNAs as a quantitative, cell-based measure of HPV infetivity. Suspension-based disinfection protocols employed ortho-phthalaldehyde (OPA), hypochlorite and alcohols.

Conclusion

Preliminary assessment of HPV infectious titers suggest that compared to common warts, clinical RRP and anogenital samples have low levels of virions present at apical surfaces. In contrast to other reports, we found HPVs from a variety of sources were susceptible to a 2.5 to 4 $\log_{10}$ reduction in infectious titer when exposed as directed to the disinfectants.

References

We conclude that HPVs are susceptible to a variety of disinfection protocols. We plan to carefully assess the infectious titers of virions present HPV-induced lesions to better determine the risk of transmission from HPV-induced warts.

References

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FC 06. Screening 2: New screening strategies country experiences

00045

PRIMARY HPV DNA SCREENING: TWO YEARS EXPERIENCE AFTER 5Y OF CO-TESTING.

09. HPV screening

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Background / Objectives

To show the effectiveness of Primary HPV testing as a cervical cancer test.

Results

The Hospital of Barbastro serves an area of 107.480 inhabitants, in the North of Spain. Target population is 24,501 women between 30 to 65 years. Patients were attended by midwives in Primary Care Centers and referred to Gynaecological Department when necessary. We screened according to the guidelines of Spanish protocol, SEGO 2010, updated in 2014. PCR hrHPV-DNA test (Cobas 4800®) was used in both periods, which delivers HPV16 and HPV18 genotypes separately and in one group a pool of other 12 hrHPV genotypes. Co-testing was carried out from 2011 to 2015 and Primary HPV testing from 2016 to 2017 with cytology triage. The Pathology Laboratory of the Hospital of Barbastro has accreditation for PAP (conventional and liquid-based cytology) and hrHPV-DNA test (cobas 4800®) according to ISO 15189.

Conclusion

Co-testing and HPV Primary testing were compared: The average number of CIN2+ detected by year was 44.2 cases vs 54.0; the mean age was 37.9±10.2 years vs 38.6±10.2; oncogenic genotypes 16/18 were 59.5% vs 51.4%; microinvasor cases were 38.9% vs 50.0%; coverage was 36.8% to 70.5% in the first period vs 73.9% to 74.4% in the second. Bivariant study showed no statistically significant differences between both periods excepting in the rate of microinvasor cases ($p<0.05$).

Sensitivity for cytology was 79.4 (IC 95%: 71.9-82.9) in the first period and 89.2% (IC 95%: 80.8-94.1) in the second.

References

The increase in the number of cases detected may be due to the coverage increase.

As there are no significant differences in demographic or pathological features between both periods it is suggested that the results of screening are the same.

Primary Care involvement allows screening for cervical cancer to be performed to everybody without additional cost.

Primary HPV testing with partial genotyping is as effective as co-testing and should replace cytology as the first (primary) test in cervical screening.

Quality Assurance in cytological screening is essential to get good results.

00065

HPV AS THE PRIMARY SCREENING TEST FOR CERVICAL CANCER: INITIAL RESULTS FROM A DANISH IMPLEMENTATION STUDY

09. HPV screening

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Background / Objectives

Within the past 10 years, several large randomized trials have shown that a test for human papillomavirus (HPV) is more sensitive than the Pap smear in detecting severe cervical cancer precursor lesions and cancer. Therefore, introduction of HPV-based screening may be very efficient in a Danish setting, as incidence and mortality of cervical cancer is higher than in most other Western European countries.

The objectives of the present study is to compare the clinical performance of human papillomavirus (HPV)-based versus cytology-based cervical cancer screening in a large, population-based implementation study in Denmark.

Results

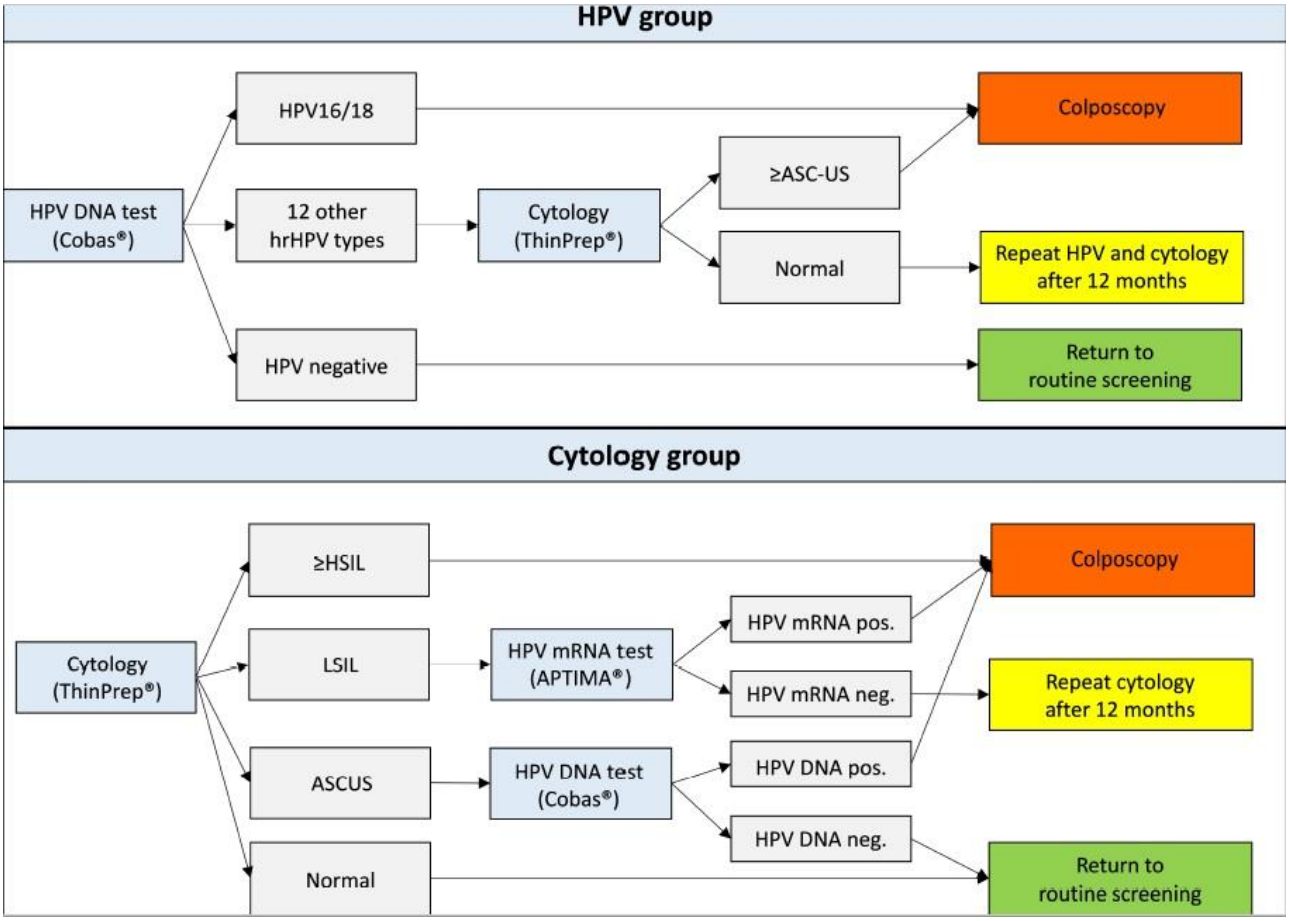
Since May 2017, a pilot implementation of HPV-based cervical cancer screening has been ongoing for women aged 30–59 years in the Region of Southern Denmark. Based on area of residence, women screened in the uptake area of Vejle Hospital are allocated to either HPV-based screening (with HPV16/18 genotyping and cytology triage) or cytology-based screening (with HPV triage for ASC-US/LSIL) (Figure 1). Here, we compare the proportion of unsatisfactory tests and referrals during the first 10 months of implementation.

Conclusion

Until March 2018, 8,851 women were screened by HPV testing and 13,359 by cytology. The age distribution (median [interquartile range]) was similar in the HPV (44 years [37–49]) and cytology (43 years [37–49]) groups. The proportion of unsatisfactory tests was lower in the HPV (0.03%, 95% CI: 0.01%–0.09%) than cytology (0.53%, 95% CI: 0.42–0.67) group. The proportion referred to colposcopy was higher in the HPV (4.0%, 95% CI: 3.6%–4.4%) than cytology (2.2%, 95% CI: 2.0%–2.5%) group. The proportion referred to repeat testing at 12 months was also higher in the HPV (5.0%, 95% CI: 4.5%–5.4%) than cytology (0.2%, 95% CI: 0.1%–0.2%) group.

References

Results for CIN2+ detection will be presented at the conference. HPV-based screening resulted in fewer unsatisfactory tests, but in this initial screening round, more women were referred to colposcopy and repeat testing than with cytology-based screening.



00192

First results of high-risk HPV screening in the cervical cancer screening programme in the Netherlands: participation, referral and detection

09. HPV screening

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Background / Objectives

In January 2017, the Dutch cervical cancer screening programme was changed from a cytomorphological screening to primary high-risk HPV DNA (hrHPV) screening for women between ages 30 and 60. This is the first time in the world, that primary hrHPV screening is nation-wide implemented in the actual population. Women can request a self-sampling set or have a cervical smear taken by their GP. Cytology testing is performed on hrHPV positive samples only. Women with cytological abnormalities (i.e. ASCUS+) are referred for colposcopy and women without

cytological abnormalities have repeat cytology testing after six months. Monitoring of the renewed screening programme is aimed at participation, hrHPV prevalence and the associated referral- and CIN rates.

Results

Screening history data was obtained from the national registry of histo- and cytopathology (PALGA), based on 426,790 primary tests performed in women who were invited in 2017 for the renewed screening programme.

Conclusion

Overall, the hrHPV positive rate was 9.0%, ranging from 21.3% (age 30) to 4.5% (age 60). Cytology was assessed in 98.8% of all hrHPV positives, leading to 32.1% referrals. The CIN2+ detection from colposcopy was 49.7%. 6.9% of the participating women opted for the self-sampling device. At a later stage, we will calculate the total referral and CIN detection rates, including those from follow-up examinations, based on full follow-up data.

References

Primary high-risk HPV DNA screening in the Netherlands leads to the detection of a large proportion of CIN2+ lesions, as a result of a substantial number of referrals for colposcopy.

00193

The longitudinal clinical performance of the RNA-based Aptima Human Papillomavirus (HPV) Assay in comparison to the DNA-based Hybrid Capture 2 HPV Test in 2 consecutive screening rounds with a 6-year interval in a Routine Screening Population of 10.000 women in Germany

09. HPV screening

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Background / Objectives

Longitudinal data on the E6/E7 mRNA-based Aptima® HPV (AHPV) assay exceeding three years in comparison to the gold standard digene Hybrid Capture® 2 (HC2) test are not available.

Results

We previously reported the cross-sectional data of the German Aptima Screening Trial (GAST) where 10,040 women were recruited and tested by liquid-based cytology, the HC2 and the AHPV assay. 411 test-positive women were followed for up to six years. In addition, 3,295 triple-negative women were screened after a median time of six years.

Conclusion

Overall 28 incident CIN3 cases were detected. The absolute risk of developing high risk HPV positive CIN3+ over six years among those women that tested negative at baseline was 2.2 (1.0-4.9) and 2.8 (1.4-5.4) per 1,000 women screened by the HC2 and the AHPV test, respectively (p=0.1094), whereas the absolute risk following a negative LBC test was 9.0 (2.7-30.4). The relative sensitivity of AHPV compared to HC2 was 94.1% for CIN3+ and the negative predictive values were 99.89 (99.77-99.95) and 99.95 (99.83-99.99), respectively.

References

Our data show that the longitudinal performance of the AHPV-test over six years is comparable to the performance of the HC2 test.

00271

HPV FOCAL 48 MONTH EXIT SURVEY: WOMEN'S REAL WORLD EXPERIENCES SURROUNDING PRIMARY HPV TESTING

09. HPV screening

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Background / Objectives

Primary HPV testing for cervical screening has been implemented or being planned in jurisdictions globally. Engaging women who participate in screening prior to implementation is essential to successful transition to HPV testing from Pap screening. HPV FOCAL, a large randomized controlled trial compared primary HPV testing every 4 years to liquid-based cytology (LBC) every 2 years. Women who completed 48mos exit screening were surveyed to assess attitudes and experiences with primary HPV screening.

Results

Women from British Columbia, Canada, aged 25-65 (n=19,009) were randomized to the control (LBC) or intervention (HPV) arms from 2008 to 2012. By 2016, 16,374 women attended exit screening with HPV and LBC co-testing at 48 months. At trial entry, participants were provided information about HPV, cervical cancer, and differences between HPV and Pap testing. In 2017, any women who had completed the 48mos exit screen and had email addresses were invited to complete a survey assessing attitudes to HPV vs. Pap testing, screening intervals, starting age for HPV screening and receipt of HPV positive results. Preliminary summary statistics are presented.

Conclusion

Of the 14,535 invites sent, 5532 (38%) completed some or all of the survey. Of those surveyed, 63% reported having an HPV test to screen for cervical cancer vs. the Pap was acceptable; 54% would be willing to have an HPV test every 4-5 years vs. a Pap every 3 years; and 69% indicated having an HPV test starting at age 30 would be acceptable. When asked about concerns regarding receiving positive results, there were statistically significant differences in distribution of responses between women who ever tested HPV positive (HPV+) vs. women who did not (HPV-); 73% of HPV- vs. 54% HPV+ indicated it would be important for them to know who gave them HPV ($p<0.001$); 25% HPV- vs. 36% HPV+ indicated having HPV would not affect the relationship with their partner ($p<0.001$). However, 70% HPV- vs. 63% HPV+ would feel comfortable telling their partner about the HPV+ result ($p=0.003$) and 36% HPV- vs. 47% HPV+ feel people may judge them negatively for having HPV ($p<0.001$).

References

In a large primary HPV screening RCT, where women were consented and provided with information regarding HPV testing, most women report HPV testing vs. the Pap would be acceptable and just over half would be willing to have HPV testing every 4-5 yrs. Women had varied concerns regarding receipt of HPV positive results. These findings can inform program planning and underscore the need for comprehensive communication and information strategies prior to implementation of primary HPV screening.

00347

Cytological triage and molecular triage with partial genotyping in HPV primary screening: comparison of data from an Italian Region (Tuscany)

09. HPV screening

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ISPRO - Florence (Italy)

Background / Objectives

HPV primary screening requires a triage for positive women and cytology is currently recommended by European guidelines. Some clinically validated HPV tests perform also genotyping and FDA approved a protocol with partial genotyping as triage: all HPV positive women are sent to immediate colposcopy, except for HPV16 and/or HPV18 negative women with normal cytology, who are invited to repeat HPV test and cytology in 1 year.

The objective of the study is to evaluate the performances of partial genotyping as triage, compared to the protocol with cytology.

Results

In Tuscany, a screening validated HPV test that performs a partial genotyping (distinguishing HPV16 and/or HPV18 positive samples from those positive to the 12 “other HPV”) is used. We considered women aged 34-64 years, participating to the HPV primary screening in Florentine area between June 2015-March 2017.

Conclusion

Among 20638 HPV tests executed with partial genotyping, 1529 (7.4%) were positive, of which 452 (29.6%) to HPV16 and/or HPV18.

Cytology triage was abnormal for 418 women (27.3%) and inadequate for 21 (1.4%), with a colposcopy referral rate (RR) of 28.7% (439/1529).

Adhesion to colposcopy was 92.5% (406/439) and 138 (34%) CIN2+ were found.

For 183/452 (40.5%) HPV16 and/or HPV18 positive women, cytology triage was abnormal/inadequate and 80/170 (47.1%) resulted CIN2+.

For 256/1077 (23.8%) “other HPV” positive women, cytology triage was abnormal/inadequate and 58/236 (24.6%) resulted CIN2+.

If we apply the American protocol, the immediate colposcopy RR would be 46.3% (708/1529).

Among the HPV16 and/or HPV18 positive women with normal cytology triage, 56/208 (26.8%) cleared the HPV infection at the 1 year recall and 23/136 (16.9%) CIN2+ were found.

References

Partial genotyping as triage, with sending HPV16 and/or HPV18 positive women to colposcopy, does not result in a change of the immediate colposcopy RR compared to cytology (29.6% vs 28.7%), unlike the American protocol (46.3% vs 28.7%, $p < 0.0001$).

Women with HPV16 and/or HPV18 infection have a greater risk of CIN2+ (47.1% vs 24.7%, $p < 0.002$), but 42% (58/138) of CIN2+ were diagnosed in “other HPV” positive women. So, partial genotyping with sending only HPV16 and/or HPV18 positive women to colposcopy cannot be used as a valid substitute triage method.

Since our protocol does not send HPV16 and/or HPV18 positive women with normal cytology triage to immediate colposcopy, we cannot compare accurately American and our protocols. We can only say that, sending also HPV16 and/or HPV18 positive women with normal cytology triage to 1 year recall instead of immediate colposcopy, we save unnecessary colposcopies for women who clear the infection in 1 year (56/708 = 7.9%).

00370

CANCER CASES IDENTIFIED IN A RANDOMIZED IMPLEMENTATION OF HPV-SCREENING IN THE NORWEGIAN CERVICAL CANCER SCREENING PROGRAMME

09. HPV screening

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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 cancer screening programme. We present detailed evaluation of the cancer cases identified.

Results

The implementation involves women in the age group 34-69 years in four Norwegian counties, counting approximately 285.000 women. In Norway, the follow-up algorithm after abnormal screening test has been more more aggressive for HPV screening than for cytology screening, referring an increased number of women to direct biopsy, and potentially earlier detection of cancers. Cancer cases, symptomatic and screening detected, are identified for both women allocated to HPV test or cytology. An early concluding cohort, including women who have had time to complete the follow-up algorithm, was used for a more unbiased comparison of the cancer cases. Descriptive analyses of screening results (cytology/HPV status/genotype), screening history, symptoms, FIGO-stadium and age of the cancer-diagnosed women are presented.

Conclusion

By March 2018, approximately 195.0000 women have been screened, half with HPV test and half with cytology. Around 107.000 women have had time to have complete follow-up, and a total of 89 cancer cases are identified in the early concluding cohort; 53 cases belonged to the cohort allocated to HPV testing (40 squamous cell carcinoma, 12 adenocarcinoma, 1 other cervical cancer type) and 36 to the cohort screened by cytology (27 squamous cell carcinoma, 6 adenocarcinoma, 3 other cervical cancer type). Majority of the cancers are diagnosed after the index test

suggested referral to colposcopy and direct biopsy. More than 50% of the women diagnosed with cancer are severe under-screeners/non-screeners. Around 75% of the cancers were related to HPV16 and HPV18, and the majority of the cancers were FIGO stadium I. Updated results will be presented.

References

Most cancer cases identified in the enrolment represent undiscovered premalignant lesions of previous screening rounds, and the actual number of cancer cases should be comparable between the two groups. Our early concluding cohort show a slight increase in the number of diagnosed cancer cases after primary HPV test, and the number of diagnosed cases should be followed closely.

00386

A 5-YEAR FOLLOW-UP STUDY OF THE DIAGNOSTIC EFFICACY USING PRIMARY hrHPV TESTING VS. LIQUIDBASED CYTOLOGY IN CERVICAL CANCER SCREENING OF WOMEN AGED 50+

09. HPV screening

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Background / Objectives

Evidence supports high risk Human Papilloma (hrHPV) testing as the primary screening tool in cervical cancer (CCU) screening. However, many studies on the effectiveness used HC2 HPV DNA as the HPV-test and compare to PAP-smears. Only few evaluated other commercially available tests in comparison with liquid-based cytologies (LBC).

We aimed to evaluate the diagnostic efficacy of hrHPV testing using Cobas® 4800 HPV DNA test (Cobas 4800) as compared to LBC in women aged 50+.

Results

Between September 1st 2011 and September 4th 2012 a consecutive sample of women aged 50+ who were routinely tested for cervical abnormalities in Central Denmark Region were included in the study. The samples were analyzed by both routine microscopy and Cobas 4800. At base-line we calculated the percentage of hrHPV positive test results and percentage of cervical precursors in the LBC. By use of the unique Danish personal identification numbers, all women were followed until December 31st 2017 in the Danish Pathology Register, which contains results of all cervical cytologies and histologies taken in any setting in Denmark. Diagnostic efficacy of LBC and hrHPV-testing was calculated using CIN2+ and CIN3+ lesions diagnosed by colposcopy and biopsy within 5 year as the golden standard.

Conclusion

A total of 4043 women of which 3.93% had ASCUS+ and 8.0% were hrHPV positive at base-line were included in the study. Of these women, 72% were registered with at least one cytology sample or cervical histology in the follow-up period. Sensitivity of CIN2+ was 46.9% (34.3%-59.8%) and 82.8% (71.3%-91.1%) for LBC and hrHPR testing, respectively. The corresponding specificities were 97.0% (96.4%-97.5%) and 93.2% (92.4%-94.0%), respectively. Using CIN3+ as the golden standard the sensitivity was 48.1% (34.0%-62.4%) for LBC and 82.7% (69.7%-91.8%) for hrHPV, while the corresponding specificities were 96.9% (96.9%-97.4%) and 93.0% (92.1%-93.7%), respectively. Two CCU diagnosed at follow-up were overlooked by each of the diagnostic procedures.

References

In this Danish population of women aged 50+ we found a much better sensitivity for CIN2+ and CIN3+ using hrHPV-testing as compared to LBC while specificity decreased slightly. Attention should be paid to the cancer outcomes.

00398

Risks of CIN3+ by cytology and human papilloma virus genotype: A risk-based approach to cervical cancer screening in Norway

09. HPV screening

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Background / Objectives

Primary human papillomavirus (HPV)-based cervical cancer screening was randomly implemented among Norwegian women aged 34 to 69 years in 2015. As an expected consequence of the more sensitive HPV test, there was an increase in the number of positive primary and follow-up tests, decreasing the overall positive predictive value in the follow-up algorithm. Risk stratification of HPV-positive women by HPV genotype may identify women who should be sent immediately to colposcopy/biopsy and those who can be safely followed-up with less invasive testing. The aim of this study was to evaluate the risk of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) among HPV-positive women, stratified by high-risk HPV genotypes (HPV16/18 vs. other high-risk (oHR)) and cytology diagnosis.

Results

Relevant information on cervical cancer screening and cervical preinvasive lesions and cancer for 94 590 women 34 to 69 years old in the HPV screening arm were extracted from four Norwegian counties from the Cancer Registry of Norway databases.

We estimated the total cumulative risk (CR) of CIN3+ among all HPV-positive women as well as for different combinations of reflex cytology results based on the current triage management of HPV-positive women. As women with atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) potentially represent the group of women most likely to yield a false-positive colposcopy (and benefit from genotyping), we also examined CIN3+ risk among this group of women.

Conclusion

We identified a total of 3059 women aged 34 to 69 years that were HPV-positive. Among this group of women, the overall cumulative risk for CIN3+ was 22.1%. For women with an ASCUS or LSIL reflex cytology result, HPV 16/18-positivity yielded a two-fold higher cumulative risk of CIN3+ compared to women with oHR-positivity (31.8% vs 15.6%, respectively). We also found that women with an HPV16/18-positive infection with NILM reflex cytology yielded a similar cumulative risk as women with an oHR infection with ASCUS reflex cytology result (i.e., 18.4% for HPV16/NILM and 17.3% for HPV18/NILM vs 15.3% for oHR/ASCUS).

References

Follow-up algorithms based on HPV16/18 positivity in women aged 34 to 69 years resulted in better discrimination of those at risk for CIN3+ development. The revised management guidelines by the Norwegian Cervical Cancer Screening Program will recommend clinical actions based on risk, rather than test-based algorithms.

00412

16/18 GENOTYPING OF PERSISTENT HR-HPV INFECTIONS WITH NEGATIVE CYTOLOGY: RESULTS FROM THE ENGLISH CERVICAL SCREENING PILOT

09. HPV screening

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Background / Objectives

In the English pilot of primary cervical screening with high risk human papillomavirus (HR-HPV) testing, HR-HPV positive women were managed as shown in Figure 1. Tests that report HPV 16/18 infections, as well as other HR-HPV infections, were used in three laboratories. This has allowed us to test whether a faster referral of women with HPV 16/18 infections and negative cytology persisting for 12 months would improve clinical outcomes such as detection of high grade cervical intraepithelial neoplasia (CIN 2+) and loss to follow-up, while keeping in balance the number of colposcopies. We compared these genotyping-based outcomes with those from the non-genotyping protocol in which a colposcopy for all women with persistent HR-HPV infections and negative cytology (HR-HPV+/cyt-) was delayed until a 24-month early recall (Figure 1).

Results

We included all 127,238 women aged 24-64 years who had been screened in the three HPV 16/18 reporting laboratories during the prevalence round before 2015. These women had at least 29 months of follow-up in the available data. For each triage protocol, we calculated the numbers of detected CIN 2+, colposcopies, and HR-HPV+/cyt- women not attending the two early recalls in the entire screened population. For the non-genotyping protocol, we estimated the 24-month outcomes for HPV 16/18 infections using attendance, persistence and detection data observed in comparable women in the pilot. The 95% CI were calculated using a parametric bootstrap.

Conclusion

With the genotyping protocol, 2869 CIN 2+ were detected as a result of 8750 colposcopies, and 2378 out of 10,810 HR-HPV+/cyt- women did not attend their early recalls. In the same 127,238 screened women, these numbers would be 2378, 8260, and 2626, respectively, with the non-genotyping protocol. Hence, the genotyping protocol increased the total number of detected CIN 2+ by 1.2% (95% CI: 0.9-1.5) and the number of colposcopies by 5.9% (95% CI: 5.0-6.9) compared with the non-genotyping protocol, and decreased the proportion of HR-HPV+/cyt- women not completing the recommended early recalls by 2.3% (95% CI: -2.5 - -2.1). These numbers were very similar across all age groups, and were robust to varying the assumptions on attendance, persistence and detection at 24 months in HPV 16/18 positive women.

References

Faster referral of HPV 16/18+/cyt- women 12 months after primary screening does not appear to improve to a meaningful extent the clinical outcomes in the English routine cervical screening programme. If anything, more rapid referral of persistently HPV 16/18+/cyt- women increased the number of colposcopies disproportionately relative to the gain in increased detection of CIN 2+, and had little effect on the completeness of follow-up.

Figure 1. Management of women in the English pilot of primary cervical screening with HR-HPV testing.

<table>
<tr><th>Time of testing</th><th>Genotyping triage protocol
(laboratories A, B, C)</th><th>Non-genotyping triage protocol
(laboratories, D, E, F)</th></tr>
<tr><td>Baseline test</td><td colspan="2">HR-HPV negative: routine recall at 3/5 years
HR-HPV positive/positive cytology: colposcopy
HR-HPV positive/negative cytology: early recall at 12 months</td></tr>
<tr><td rowspan="2">Early recall at 12 months</td><td colspan="2">HR-HPV negative: routine recall at 3/5 years
HR-HPV positive/cytology positive: colposcopy</td></tr>
<tr><td>HPV 16/18 positive/cytology negative: colposcopy
Other HR-HPV positive/cytology negative: early recall at 24 months</td><td>HR-HPV positive/cytology negative: early recall at 24 months</td></tr>
<tr><td>Early recall at 24 months</td><td colspan="2">HR-HPV negative: routine recall at 3/5 years
HR-HPV positive: colposcopy</td></tr>
</table>

00058

FIVE-YEAR RISK OF CERVICAL PRECANCER FOLLOWING P16/KI-67 DUAL STAIN TRIAGE OF HPV-POSITIVE WOMEN

13. Screening methods

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Background / Objectives

Human papillomavirus (HPV)-based screening requires triage to avoid unnecessary colposcopy referral while maintaining high sensitivity for cervical precancer. Triage with p16/Ki-67 dual stain (DS) has shown high sensitivity and specificity for detection of cervical precancers; however, prospective studies evaluating the long-term risk of cervical precancer following p16/Ki-67 testing are lacking. Such studies are critical to determine how long a negative DS result indicates a low risk of precancer in order to establish optimal screening intervals. We evaluated the longitudinal performance of p16/Ki-67 DS triage for detection of cervical precancer in a clinical population of over 1,500 HPV-positive women with up to 5 years of follow-up in the context of clinical management thresholds.

Results

1,549 HPV-positive women undergoing screening with HPV/cytology (SurePath) co-testing were enrolled in 2012 at Kaiser Permanente Northern California. Histological endpoints were ascertained from the clinical database through 2017. We estimated 5-year cumulative risks of cervical intraepithelial neoplasia grades 2 or worse (CIN2+) or grades 3 or worse (CIN3+) by baseline p16/Ki-67 DS and cytology at yearly intervals using Logistic Weibull models. Risks were compared to clinical management thresholds for colposcopy referral and a one-year return interval.

Conclusion

p16/Ki-67 DS-positivity predicted significantly higher cumulative 5-year risks of CIN2+ compared to abnormal cytology ($p<0.05$). p16/Ki-67 DS-negative women had

significantly lower 5-year risks of CIN2+ compared to women with normal cytology ($p<0.05$). Similar results were observed for CIN3+. In p16/Ki-67 DS-negative women, the risks of both CIN2+ and CIN3+ remained below the colposcopy referral threshold for all 5 years, and crossed the one-year return threshold at 3 years.

References

Triage with p16/Ki-67 dual stain provides better long-term risk stratification compared with cytology over 5 years. The low risk of cervical precancer in women testing p16/Ki-67 DS-negative permits safe extension of follow-up intervals for 3 years.

00329

DEVELOPMENT OF EVIDENCE-BASED GUIDELINES FOR FOLLOW UP OF WOMEN TREATED FOR CERVICAL INTRAEPITHELIAL NEOPLASIA GRADE 2 OR 3 (CIN2/3) IN ITALIAN SCREENING PROGRAMS.

13. Screening methods

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Background / Objectives

The Italian National Guidelines System of the National Health Institute adopted the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) methodology for the development of guidelines. The Italian Group for Cervical Cancer Screening (GISCi) promoted the update of recommendations for post CIN2/3 treatment follow up.

Results

A multidisciplinary panel including all the professionals involved in cervical cancer screening programme and CIN treatment was set up. The GRADEpro online tool was used for: defining and prioritizing clinical questions framed in PICO (Population Intervention Comparator Outcomes); defining and scoring outcomes as critical, important or not important; synthetizing results of the systematic reviews in Evidence to Decision tables and to grade recommendations as strong, conditional (in favour or against) and conditional either the two. Recommendations for six PICOs were published in 2018 (www.gisci.it) while systematic reviews for remaining PICOs are starting.

Conclusion

Sixteen questions were included in the scope and framed in PICOs: 3 about the test to be used (Pap, HPV-DNA or Pap+HPV co-testing or co-testing+colposcopy); 1 about the number of follow up episodes before returning to screening; 6 about the timing of episodes; 3 about the use of diagnostic leep in women with positive follow up test and negative colposcopy; 1 about use of typing test to distinguish persistent

from new infections; 1 about HPV vaccination in treated women. The panel recommended HPV test or co-testing (conditional), but not Pap as follow up test (strong). Colposcopy can be added, but only for assessment of surgical outcomes (conditional either yes or not). Two episodes instead of one, before referring women to regular screening, should be preferred (conditional), because even if some observational studies showed a risk comparable to the general population after one negative co-testing, other studies gave different results. The first episode should be after 6 months (vs. 12) after treatment (strong), in order to avoid progression of undiagnosed prevalent invasive cancers; the interval between first and second episode could be either 6 or 12 months (conditional).

References

Defining evidence based follow up protocols is challenging since no experimental evidence comparing different options is available. Clinical questions should be framed to allow the use of indirect evidence from natural history of the disease and observational studies. GRADE offered a framework to evaluate indirect evidence particularly in accuracy of diagnostics even if dichotomous questions are not the most efficient frame to answer question about frequency and intervals.

00333

SENSITIVITY AND POSITIVE PREDICTIVE VALUE OF HPV E6/E7 mRNA OVEREXPRESSION ASSAY AS TRIAGE TEST FOR HPV POSITIVE WOMEN.

13. Screening methods

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Background / Objectives

Screening by HPV-DNA test showed to be more effective than Pap test in preventing cervical cancer, but the large proportion of women who test positive makes necessary a triage test to refer women to colposcopy.

New Technology in Cervical Cancer 2 (NTCC2) is a randomized trial testing biomarkers for triaging HPV positive women; here we compare accuracy of cytology and an E6/E7 mRNA overexpression assay targeting 14 types (16/18/31/33/35/39/45/51/52/56/58/59/66/68).

Results

More than 41,000 women were recruited in 5 Italian centres (Florence, Veneto, Umbria, Turin, Trento); data from the first 3 centres are included in these analyses. Women were tested with HPV DNA test). Those positive were triaged with cytology and tested for E6/E7 mRNA and other biomarkers. Women with positive cytology were referred to colposcopy, those with negative cytology were randomised to immediate colposcopy or to 1 year HPV re-testing. Women will be followed up until the next screening round (at least for 5 years). All women were tested for E6/E7 mRNA overexpression at baseline. Here we report sensitivity for CIN2+ (at recruitment or at 1 year follow up) of cytology (at ASCUS+ threshold) and of E6/E7 mRNA assay, the rate of positivity, and the estimated positive predictive value (PPV) at baseline.

Conclusion

33,388 women aged 25-64 years have been recruited and 2354 (7.1%) were HPV-DNA positive. 2333 cases have both mRNA and cytology results; 1617 (69%) were positive for mRNA and 651 (28%) for cytology. Cumulatively, 125 CIN2+ (63 CIN2 and 62 CIN3) were found; sensitivity was 96% (120/125; 95%CI 91-99) and 69% (86/125; 95%CI 60-77) for mRNA and cytology, respectively; the estimated PPV was 10% for mRNA (120/1215; 95%CI 8-12) and 15% for cytology (86/581; 95%CI 12-18).

Among the 788 HPV-positive/cytology-negative women randomized to immediate colposcopy, 22 CIN2+ (14 CIN2 and 8 CIN3) were detected (2.8%), while among the 894 randomized to 1 year HPV re-testing, 754 completed the follow up and 17 CIN2+ (10 CIN2 and 7 CIN3) were found (2.3%)(RR 0.81;95% CI 0.43-1.52). The 5 mRNA-negative CIN2+ were found after immediate colposcopy; 2 (1 CIN2 and 1 CIN3) were cytology positive and 3 (all CIN2) were cytology negative.

References

E6/E7 mRNA assay would refer to immediate colposcopy more than 2/3 of HPV-DNA-positive women, but its sensitivity is very high and could allow longer interval for retesting. Similar detection of CIN2+ after immediate colposcopy and 1-year HPV re-testing suggests limited regression, if any, of CIN2+ in one year but current confidence intervals don’t allow excluding a relevant one. Quick regression of mRNA negative CIN2+ could explain the lack of mRNA negative CIN2+ observed in the re-testing arm if confirmed on larger scale.

00037

SCREENING OUTCOME AFTER HPV-VACCINATION IN DENMARK

22. Cervical neoplasia

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Background / Objectives

In Denmark, free HPV-childhood vaccination was offered first to girls born in 1993. In 2016, these girls turned 23 years old and were invited for the first time to cervical screening. The purpose of the study was to determine the effect of this population-based HPV-vaccination.

Results

We followed the closed cohort of women born in 1993, and present in Denmark both when offered HPV-vaccination in 2008 and when invited to screening for the first time in 2016. For comparison we followed a similarly closed cohort of women born in 1983, and present in Denmark when invited to screening for the first time in 2006. Outcome of first screen was determined by linkage to the Danish National Pathology Register. We calculated Relative Risk (RR) and 95% Confidence Intervals (95% CI) for 1) cytology being atypical squamous cells of undertermined significance (ASCUS); ASCUS+; or high grade squamous intraepithelial lesion (HSIL); and for histology being cervical intraepithelial noeplasia (CIN); or CIN3.

Conclusion

In both cohorts, around 60% of women had been screened; around 60% had completed high school; the average age of sexual debut was 16 years; but percent daily smokers at age 15 had decreased from 21% for women born in 1983 to 10% for women born in 1993. In the closed 1993 cohort, 92% of women had received at least one dose of the HPV-vaccine before invitation to screening, while this was 0% for the closed 1983 cohort. For cytology, RR of ASCUS+ was 1.04 (95% CI 0.96-1.12), reflecting the combination of a statistically significant decrease in HSIL; RR 0.60 (95% CI 0.5-0.7) and an increase in ASCUS; RR 1.4 (95% CI 1.2-1.6). In Denmark, women with HSIL are referred directly for colposcopy with biopsy, while women with ASCUS are referred for repeated cytology testing in 6 months. We are currently

analysing these follow-up data, which require a longer observation period that the first screen cytology data.

References

Women in the HPV-vccinated cohort had 40% less HSIL than women in the non-vaccinated cohort. These women are spared referral to colposcopy with biopsy. The observed increase in ASCUS in the vaccinated as compared with the non-vaccinated cohort can probably be explained by the change over time from predominantly conventional to entirely, and mostly SurePath, liquid-based cytology. As we compared entire birth cohorts of vaccinated and non-vaccinated women, our study was free from the selection bias which normally affects comparisons between participants and non-participants in HPV-vaccination. The final answer to the effectiveness of the population-based HPV-vaccination in Denmark will come from the histology data we are currently analysing.

References

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00094

CURRENT STATUS OF CERVICAL CANCER SCREENING PROGRAMS AND HPV VACCINATION IN SOUTHEAST EUROPEAN COUNTRIES

22. Cervical neoplasia

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Background / Objectives

The Southestern Europe HPV Forum is a non-profit and non-governmental organization of southeast European countries established in 2018 with a goal to promote the research of all aspects of human papillomavirus (HPV) infection, to study diseases caused by HPV and help to implement and/or improve primary and secondary prevention programs of cervical cancer and other HPV-related tumours.During the transition period, most of the south-eastern Europe countries experienced significant changes in the healthcare system, especially in the area of medical general practice. Privatization waves have significantly influenced health standards and the availability of health care. Part of the health care has been significantly improved. However, one part of health care has maintained the previous standards or there has been a weakening, especially in the case of diseases and conditions that have a public health significance. Among the world’s leading causes of morbidity and mortality, cervical cancer have understandably been the primary focus of research and development and the dominant motivation for international cooperative efforts at prevention and control.

Results

Successfully organised, population-based cervical cancer screening programmes have not yet been implemented in most southeast European countries despite the greatest burden of cervical cancer. Effective national organized screening in Slovenia

started in 2003, and incidence of cervical cancer decreased since then by 44%. However, the last four years the incidence of cervical cancer recorded the plateau. HPV vaccine coverage in Slovenia is about 55%. In Croatia, the organized cervical cancer screening started in 2012. Through 3 years of program, the incidence of disease was reduced by 18%. Unfortunately, in Croatia HPV vaccination coverage is less than 10% at the state level. In Bulgaria, Romania, Serbia, Montenegro and FYR Macedonia the implementation of organized cervical cancer prevention programmes are in progress, current standard is opportunistic screening with poor coverage. Also, there are no reliable data on HPV vaccination uptake. National-based and country-tailored screening solutions need to be established, using experiences of successful screening programmes in the region.

References

The reframed programs of cervical cancer prevention will include strategic combinations of at least two major components: extension and advancement of existing screening programs using HPV-based technology and implementation of national gender-neutral HPV vaccination in all countries. Understanding the nature of prevention tools, how to use them and how to evaluate their impact is a pressing social demand for the scientific, medical and public health communities.

FC 07. HPV Testing

00009

COMPARISON AND BENEFITS OF FULL GENOTYPING OF ALL 14 ONCOGENIC HPV TYPES USING INNO-LIPA® EXTRA II VERSUS GENOTYPING HPV-16, HPV-18 INDIVIDUALLY AND POOL DETECTION OF 12 OTHER HIGH RISK HPV WITH COBAS 4800® AMONG IRANIAN WOMEN

08. HPV testing

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Background / Objectives

Virtually all Cervical cancers are caused by persistent infection with oncogenic HPV types.HPV 16 and18 Combined are responsible for causing almost 70-75% of all cervical cancers,but the remaining 25-30% of cervical cancers are caused by at least 12 other HR-HPV genotypes;Therfore other oncogenic HR-HPV genotypes demonstrate risk as well for cervical dysplasia and cancer.The aim of this study was to assess whether full genotyping of all 14 oncogenic HR-HPV types are more beneficial.

Results

We examined 1811 women of 25-65 years whom attended DML Medical Center for cervical cancer screening in Mashhad,Iran from June 2017 until July 2018.All participants,before collection of cervical samples,completed a questioner which included their age,Marital status,parity,use of tobacco as well as oral contraceptive pill.All Cervical samples,collected between June to October 2017,were initially tested with cobas 4800 for HR-HPV which detects HPV 16, HPV 18 separately and 12 other HR-HPV types as a group,followed by retesting the samples by INNO-LiPA Extra II which detects 32 HPVs individually;19 HR-HPV as well as 13 LR-HPV types.Both assays were carried out according to manufacturer’s instructions.In the second phase,all positive patients for HR-HPV with Cobas 4800 were asked to return after 9

months from the initial test to assess regression,persistence or replacement of their HPV infection.

Conclusion

From 1811 samples,15.5% (281/1811) were positive for HR-HPV with cobas and 16.8% (305/1811) were positive by INNO-LiPA.WIth Cobas,the most prevalent HR-HPV were HPV-16(28.8%),HPV-18(8.9%),and other HR-HPV(62.2%) and with INNO-LiPA were HPV-16(25.5%),HPV-18(7.5%) and other HR-HPV(66.8%).No major discordant finding were seen between Cobas and INNO-LiPA regarding HPV16 (81 vs. 78) samples and HPV18 (25 vs. 23) samples respectively,however we observed more Positive other HR-HPV by INNO-LiPA(204 vs. 175) since it detects more HR-HPV(19 HR vs.14 HR) per sample.Rescreening positive patients after 9 months,we again detected no discordant finding between Cobas and INNO-LiPA for HPV 16(28 vs. 25), and HPV 18 (8 vs. 6) respectively,but in regard to other HR-HPV,we observed 61 positive HPV by Cobas and 73 positive HPV by INNO-LiPA,but majority of other HR-HPV by INNO-LiPA were replaced by different types since INNO-LiPA detects all other HR-HPVs individually,whereas cobas detects them as a group.

References

This study shows INNO-LiPA Extra II has more advantages since it can fully genotype all 14 HR-HPVs separately and can demonstrate if HPV infection by other HR-HPV types has been replaced, regressed or is still persistent which can help clinicians in clinical follow-up studies of patient, prevention strategies,vaccine efficacy and development of new therapies.

00041

KERATIN-BASED SAMPLE VALIDITY TESTING IMPROVES TRIAGE OF HPV 16/18/45 POSITIVE WOMEN USING hrHPV E7-ONCOPROTEIN TESTING

08. HPV testing

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Background / Objectives

HPV-tests based on the detection of viral oncoproteins are suitable for implementation as a triage method to colposcopy for hrHPV-positive women. The diagnostic capabilities, however, may be limited without assessment of specimen validity to reduce false negative results of these tests. Here we describe the combination of two novel assays: detection of high-risk HPV E7 proteins plus detection of Keratins 5/8/18 from potential basal squamo-columnar junction target cells.

Results

Two sandwich ELISAs – *recom*Well HPV 16/18/45 and *recom*Well Keratin 5/8/18 - were developed for detection of hrHPV E7-oncoproteins and basal keratinocytes. Cervical samples were obtained in PreserveCyte medium from 2637 women who participated in the PIPAVIR studies. All samples were characterized by cytology and HPV-genotyping; E7 and Keratin measurements were performed.

Conclusion

Keratin detection was analyzed and the proportion of invalid samples with results below cutoff was determined. An increase of invalid samples was found in CIN1+ samples (12.4%) in comparison to HPV-negative samples with normal cytology (4.1%).

Sensitivity, specificity, PPV and NPV for *recom*Well HPV 16/18/45 were calculated with HPV 16/18/45 positive samples. An increase in sensitivity of 8.9% was achieved for the CxCa-group when calculated with regard to the results of the *recom*Well Keratin 5/8/18 validity testing. On the contrary, specificity (97.9%/98.0%), PPV (19.5%) and NPV (99.9%) remained constant when compared to the results without validity testing.

	E7 result [included]	**Keratin result [included]**	**E7 result [excluded]**	**Keratin result [excluded]**	**Sens. [Normal Cytology]**	**Sens. [CIN1]**	**Sens. [CIN2]**	**Sens. [CIN3]**	**Sens. [CIS/CxCa]**	**Spec. [CIS/CxCa]**	**PPV [CIS/CxCa]**	**NPV [CIS/CxCa]**
without validity testing	all samples	all samples	-	-	10/120 8.3%	7/45 15.6%	1/28 3.6%	15/31 48.4%	8/10 80.0%	1642/1675 98.0%	8/41 19.5%	1642/1644 99.9%
with validity testing	positive negative positive	positive positive negative	negative	negative	10/116 8.6%	7/37 18.9%	1/25 4.0%	15/29 51.7%	8/9 88.9%	1571/1604 97.9%	8/41 19.5%	1571/1572 99.9%

References

Our results support the detection of hrHPV E7 oncoprotein with *recom*Well HPV 16/18/45 as a method for triage to colposcopy for HPV16/18 positive women in a screening program based on primary HPV screening with HPV16/18 genotyping. Sample validity was analyzed and differences were found in the proportion of valid samples between healthy women and those which developed HPV-induced dysplasia (CIN2+). Validity testing of cervical samples with *recom*Well Keratin 5/8/18 in combination with HPV testing is therefore mandatory to to increase sensitivity for disease with a maximum of specificity, PPV, and NPV.

References

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00074

INTERNATIONAL QUALITY ASSURANCE OF HPV DNA GENOTYPING SERVICES: THE 2017 GLOBAL HPV DNA PROFICIENCY STUDY

08. HPV testing

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Background / Objectives

The International Human Papillomavirus (HPV) Reference Center supports quality and order in HPV research and diagnostics. Notably, the center assigns HPV type numbers to novel HPV types, maintains a reference clone repository, and issues international proficiency panels for HPV genotyping. This international HPV DNA genotyping study issued in 2017 assesses the proficiency of the different HPV typing assays used routinely in laboratories worldwide as well as the performance of the laboratory.

Results

Participating laboratories were asked to perform HPV typing using one or more of their usual assays on 41 coded samples composed of purified whole genomic plasmids of sixteen HPV types (HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68a and 68b) in a background of human cellular DNA.

Proficient typing requires detection in both single and multiple infections of 50 International Units of HPV 16 and HPV 18 DNA/ 5µl and 500 genome equivalents in 5 µl for the other types, with at least 97% specificity.

Conclusion

The 2017 proficiency study had 115 participating laboratories from all over the world: More than 20 different assays were used to analyse the panel and results from 141 datasets were reported. Participating laboratories were public health laboratories, research laboratories, diagnostic test manufacturers and vaccine companies. We see an improvement in completely proficient laboratories over time from 32% in 2008 to 67% in this year. 70 % of the datasets reported no false negative result in 2017

compared to 43 % in 2008. There is a decrease in not proficient tests with more than 1 false positive result from 36% in 2008 to 14% in 2017.

References

A continuing and increasingly popular global proficiency program promotes comparability and reliability of HPV genotyping assay performance worldwide.

00205

PERFORMANCE OF THE ONCLARITY™, COBAS® AND HYBRID CAPTURE II HPV ASSAYS ON PRESERVCYT® SPECIMENS WITH PANEL-ADJUDICATED HISTOLOGY

08. HPV testing

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Background / Objectives

The BD OnclarityTM HPV Assay U.S. PMA clinical trial enrolled 33,858 subjects and the assay obtained FDA approval in February, 2018 for primary screening, ASC-US reflex, and co-testing claims using BD SurePath™ media (1). A subset of women are being followed in a three year longitudinal study. The trial design includes collection of a Hologic PreservCyt® vial. Residual PreservCyt specimens were stored at -20°C. Here we report the split sample performance of the BD Onclarity, Roche cobas, and Qiagen HC2 using a subset of these archived longitudinal specimens with reference to the panel adjudicated histology results.

Results

A convenience subset of 511 residual PreservCyt specimens were removed from -20°C storage. 161 subjects had a final adjudicated diagnosis of CIN2+, while the remainder, n = 350, had a diagnosis of < CIN2. Samples were aliquoted prior to blinded testing by cobas and HC2 (third party CLIA laboratories running respective FDA-approved tests) or Onclarity (BD Diagnostics), per the manufacturers' instructions. The combined results represent approximately one fourth of the total number of diseased patients in the U.S. PMA trial.

Conclusion

Test	FP	TP	FN	TN	Total*	Sensitivity (95% CI)	Specificity (95% CI)	Positivity (95% CI)
Onclarity	86	93	8	290	477	92.08% (85.14%,95.93%)	77.13% (72.62%, 81.09%)	37.53% (33.3%, 41.95%)
cobas	92	90	11	284	477	89.11% (81.54%, 93.81%)	75.53% (70.94%, 79.6%)	38.16% (33.91%, 42.59%
HC2	92	94	7	284	477	93.07% (86.38%, 96.6%)	75.53% (70.94%, 79.6%)	38.99% (34.72%, 43.44%)

*496/511 patients had valid adjudicated histology results, which included 104 CIN2+ (CIN2 or >=CIN3) and 392 <CIN2 (Negative or CIN1);
477/496 had valid results for all three tests, which included 101 CIN2+ and 376 <CIN2 specimens.

	Sensitivity		Specificity		Positivity	
Test Comparison	**Difference (95% CI)**	**p-value**	**Difference (95% CI)**	**p-value**		**p-value**
Onclarity vs. cobas	2.97% (-5.43%, 11.61%)	0.631	1.6% (-4.49%, 7.68%)	0.668	-0.63% (-6.78%, 5.52%)	0.894
Onclarity vs. HC2	-0.99% (-8.88%, 6.79%)	1	1.6% (-4.49%, 7.68%)	0.668	-1.47% (-7.62%, 4.70%)	0.689

There was no significant difference in clinical performance between assays. All three assays exhibited good sensitivity for CIN2+ disease.

References

The BD Onclarity HPV Assay performed equivalently to two other FDA-approved assays in split sample testing using adjudicated histopathology endpoints.

References

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00229

Buffer and time dependent HPV DNA stability in Colli-Pee® collected FV urine

08. HPV testing

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Background / Objectives

Great interest has been directed towards the use of first-void (FV) urine as a liquid biopsy for high-risk human papillomavirus DNA testing. The positive effect of a conservation buffer on the stability of HPV DNA has been reported previously [(1)]. In this study we examined the impact of different buffer (UCM) conditions and storage time points on the detection of HPV plasmid DNA and human DNA (hDNA) in FV urine samples.

Results

Eight volunteers provided a Colli-Pee® (Novosanis) collected FV urine sample that was aliquoted to test four buffer conditions (no buffer, UCM, fresh UCM) during four different storage time points (72h at room temperature (RT), 7 days RT, 7 days RT + 7 days frozen, 14 days RT). FV urine samples were spiked with HPV16 plasmid DNA and HPV DNA analysis was performed using the Cobas 6800 (Roche). Statistical analysis was performed using JMP Pro 13.

Conclusion

78% (n=25/32) of the FV urine samples without UCM became HPV plasmid DNA negative throughout the different time points. By contrast only 3% (3/93) of the samples with buffer became HPV DNA negative (n=1/3 fresh UCM; n=2/3 UCM), all after 14 days storage at RT. A faster decay of HPV DNA than hDNA was observed. There were no significant differences in HPV DNA in cycle threshold (Ct)-values observed between the buffers used, whereas significant different Ct-values were detected between samples stored for 72h at RT versus (i) 7 days at RT + 7 days frozen at -35°C and (ii) 14 days at RT (Wilcoxon matched pairs signed rank test, $p<0.05$).

References

Significant statistical differences were only observed between samples with buffer and without buffer, no significant differences were observed between the different buffers used. FV urine with UCM appeared to be stable with respect to HPV plasmid DNA for up to 7 days at RT, however after 14 days significant different Ct-values were observed. In addition, hDNA is not an ideal confirmation of good sample storage or processing because HPV DNA seems to decay faster than hDNA, which is in line with previous research.

References

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00238

ONCLARITY PERFORMANCE IN HPV DNA DETECTION OF FORMALIN FIXED PARAFFIN EMBEDDED CERVICAL SAMPLES

08. HPV testing

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Background / Objectives

The causal role of a persistent infection of HPV in cervical pre-neoplastic lesion and carcinoma development has been well established. Diagnosis of HPV infection is usually performed from cervical liquid based cytology specimens (LBC), but these often contain a large amount of HPV genotypes infections, most of which are thought to be transient infections. For this reason, the HPV tests have been developed for cytological samples and clinical cut-off of validated HPV tests is CIN2+ detection. Identification of HPV DNA in cervical tissue could be important for understanding cervical carcinogenesis and for evaluating cervical cancer management. The aim of the study is to evaluate the performance of BD Onclarity HPV test genotyping method on formalin fixed paraffin embedded (FFPE) cervical specimens compared to genotyping results from cytological samples.

Results

: FFPE specimens from women surgically treated for a cervical intraepithelial lesions (CIN) histologically confirmed at the European Institute of Oncology (IEO), Milan, from September 2012 to June 2013 were retrieved from the archives of the Department of Pathology of IEO. A series of 4-µm-thick tissue sections was cut from each paraffin block. The first and last sections were stained with hematoxylin and eosin (H&E) to confirm the histological diagnosis. FFPE and LBC specimens were genotyped using the same extended genotyping Onclarity assay.

Conclusion

Preliminary data on 37 samples (10 CIN1, 12CIN2 and 15 CIN3+) show an overall agreement of 92% for HPV status between FFPE Onclarity samples versus LBC samples. In case of concordance, at least one of the genotypes detected in LBC sample was found in the tissue sample with HPV 16 genotype the most prevalent (41%).

References

Our data demonstrate that there is a very good concordance between HPV genotypes found in cytological and tissue samples, suggesting that the Onclarity method could also be used to detect HPV in tissue samples and that the HPV genotype detected in FFPE samples is one of the HPV detected in cytological samples, supporting the thesis that a lesion is caused by one HPV genotype.

00344

HC2® vs COBAS® 4800: COMPARISON OF CLINICAL AND ANALYTICAL PERFORMANCES OF TWO CLINICALLY VALIDATED TESTS FOR HPV PRIMARY SCREENING OF CERVICAL CANCER

08. HPV testing

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Background / Objectives

In Italy, HPV screening program is started in 2013. In Tuscany Region, it was implemented in women of 34-64 years and two HPV tests, both validated for screening according to European guidelines, were used: HC2® (Qiagen®) until 2016 and Cobas® 4800 (Roche®) from 2016.

The objective is to analyse the impact of the transition from HC2® to Cobas® on HPV screening, comparing clinical and analytical performances.

Results

The study was conducted on two levels:

1) on the same population, comparing screening indicators (of baseline and 1 year recall) before and after passing from one test to another; we considered women participating to the HPV screening program of Florentine area, collected in ThinPrep® (Hologic®) from June 2015 to March 2017;

2) on the same set of samples (HC2® positive retested on Cobas®); the discordant samples were typed on L1 by a Reverse Line Blot method (AB Analitica®) and analysed on a screening assay that targets E6/E7 (BD Onclarity™).

Conclusion

1) On the same population, HPV positivity was 9.8% for HC2® samples and 7.4% for Cobas® ones ($p < 0.0001$). The rates of abnormal/inadequate cytology triage and of adhesion to colposcopy were comparable in the two groups. For women of HC2® group, compared to those of Cobas® group, at immediate colposcopy we found that

the CIN2+ PPV (23.8% vs 34%, p < 0.001) and the rate of normal colposcopies/histologies (44.1% vs 34.2%, p< 0.004) were different with statistical significance. At 1 year recall, all tests were analysed by Cobas® and HPV positivity was respectively 40.7% and 62.2% (p< 0.0001), while CIN2+ PPV was comparable between the two groups.

2) About HC2® positive samples retested on Cobas®, 32.4% resulted HR-HPV negative to the re-test, of which 82.1% had normal cytology. Discordant samples were typed on L1: 7% resulted positive to the 12 HR-HPV and 43.8% HPV negative; 49.2% were positive to HPV types different from the 12 HR-HPV (2/99 CIN3). Among discordant samples resulted negative to the 12 HR-HPV on L1, 14.5% were positive on BD Onclarity™.

References

The use of HC2® as primary screening test, compared to Cobas®, has registered:

- at baseline: greater HPV positivity, similar colposcopy referral rate, lower CIN2+ PPV, higher frequency of normal colposcopies/histologies;

- at 1 year recall (all samples analysed with Cobas®): lower HPV positivity, comparable CIN2+ PPV.

Furthermore, Cobas® is resulted more specific than HC2®: overall, 79.1% of discordant samples resulted HR-HPV negative by typing on L1 or E6/E7. However, the major analytical specificity of Cobas® has determined the non identification of 2 CIN3, detected by HC2® thanks to its cross-hybridization, that thus increases its clinical sensibility.

00352

HPV TYPE-SPECIFIC AGREEMENT BETWEEN LINEAR ARRAY HPV GENOTYPING TEST, ANYPLEX II HPV28 AND 21 HPV GENOARRAY WITHIN THE VALGENT-3 FRAMEWORK

08. HPV testing

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Background / Objectives

To assess human papillomavirus (HPV) type-specific concordance between Linear Array HPV Genotyping Test (Linear Array), Anyplex II HPV28 Detection (Anyplex) and 21 HPV GenoArray Diagnostic Kit (GenoArray) within the third VALGENT study panel (VALGENT-3).

Results

The VALGENT 3 panel comprises samples obtained from women aged 25-64 years attending the national organized cervical screening program in Slovenia (screening population), enriched with 300 cytological abnormal samples (100 ASC-US, 100 LSIL, 100 HSIL). Type-specific agreement for 12 high-risk (hr) HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 6 low-risk HPV types (HPV6, 11, 42, 53, 66, and 68) common to all three tests were assessed by Cohen’s kappa statistic (κ) and McNemar statistics on a total of 1,600 samples.

Conclusion

Excellent to good agreement between Linear Array, Anyplex and GenoArray was observed for 12 hrHPV types overall and for all individual HPV types, except for HPV42 and HPV68. Whilst Anyplex and GenoArray were in good agreement with each other (κ=0.792 for HPV42 and κ=0.765 for HPV68), they were in fair agreement with Linear Array (κ=0.291 and 0.336 for HPV42 and κ=0.313 and 0.281 for HPV68, respectively). Positivity rate for hrHPV overall and for HPV6, -31, -39, -42, -45, -53, -56, -66, and -68 determined by Anyplex and for hrHPV overall and for HPV31, -42, -45, -51, -56, -59, and -68 determined by GenoArray was statistically significantly higher than that determined by Linear Array (all pMcN<0.05). In addition, positivity rate for HPV42, -51, -53, -59, -66, and -68 was significantly different between

Anyplex and GenoArray. Nevertheless, in the total study population overall agreement between Anyplex, GenoArray and Linear Array was consistently above 96.5% (ranging from 96.5-100.0%) for overall and all individual 18 HPV types assessed.

References

Anyplex, GenoArray and Linear Array showed excellent agreement for the majority of HPV genotypes assessed within VALGENT-3.

00438

Clinical validation of the Liferiver Harmonia HPV assay using the VALGENT-4 framework

08. HPV testing

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Background / Objectives

To evaluate the clinical performance of the Liferiver Harmonia HPV assay (Harmonia) using the international Validation of HPV Genotyping Tests (VALGENT-4) framework.

Results

The VALGENT-4 panel consisted 1,297 samples from women aged 30-59 years who participated in the Danish cervical cancer screening program (998 consecutive samples from routine screening enriched with 299 cytological abnormal samples (100 ASCUS, 100 HSIL and 99 HSIL). Harmonia identifies separately HPV16 and HPV18 and 12 other hrHPV types in aggregate. Disease was defined as histologically confirmed CIN2+ (n= 119 [denominator for sensitivity]), whereas two consecutive negative cytology results were accepted as proxy for non-disease (n=898 [denominator for specificity]). Performance relative to GP5+/6+- PCR-LMNX (standard comparator test) was assessed by a non-inferiority test. Intra/inter-laboratory reproducibility of Harmonia was performed in a subset of 500 randomly selected samples. The benchmarks for acceptable HPV DNA tests in cervical cancer screening are: 0.90, 0.98 and 0.87 for relative sensitivity, relative specificity and inter/intra-reproducibility, respectively.

Conclusion

The relative sensitivity and specificity of Harmonia vs GP5+/6+-PCR-LMNX was 1.06 (95% CI, 1.02-1.11; pn.inf < 0.001) and 0.97 (95% CI, 0.85-0.90; pn.inf = 1.000), respectively. Application of an optimised a-posteriori cut-off for HPV16, HPV18 and other hrHPV types in aggregate led to the relative values of 1.04 (95% CI, 0.99-1.08;

pn.inf < 0.001) and 1.01 (95% CI, 0.99-1.03; pn.inf = 0.002), respectively. The assay showed good intra/inter-laboratory reproducibility (reproducibility ≥ 95%).

References

At the predefined cut-off, Harmonia HPV was statistically more sensitive but less specific than the GP5+/6+-PCR-LMNX for the detection of CIN2+. A posteriori cut off optimization was employed, and when the optimised cut-offs are applied, Harmonia fulfilled international criteria for use in cervical cancer screening.

00462

IS CO-TESTING WITH A 3-TYPE HPV MRNA TEST A BETTER STRATEGY FOR WOMEN 21-29 YEARS THAN CYTOLOGY ALONE?

09. HPV screening

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Background / Objectives

Women in their 20s are advised to get Pap/LBC, not HPV DNA tests because of the high positive rate. The prevalence of CIN3+ within this age group is high. Despite organized cytology screening, cervical cancer incidences are increasing in young women addressing the need of accurate tests. A 3-type HPV mRNA test is more specific than a 14-type HPV DNA test, and may be used in young women. We wanted to estimate the test positive rates and risk of CIN3+ in women 21- 29 years using cytology and a 3-type HPV mRNA E6/E7 test detecting genotypes 16, 18 and 45, the three most prevalent genotypes in cervical cancer.

Results

In 2013-2017, 15,428 women aged 21-29 years attended screening at the University Hospital of North Norway. 9,656 (62.6%) were co-tested using a 3-type HPV E6/E7 mRNA test (PreTect SEE; direct genotyping 16, 18 and 45). The women were followed-up until July 2018. We used histologically confirmed CIN3+ as study endpoint.

Conclusion

During follow-up, we detected CIN3+ in 3.2% (487/15,428) women. The positive rates at baseline for ASC-US+, ASC-H+ and HPV mRNA were 24.8% (3,819/15,428), 5.2% (805/15,428) and 9.7% (935/9,656). For co-testing, the test positive rates were 28.2% (2,725/9,656) and 12.0% (1,160/9,656) with cut-off ASC-US+ and ASC-H+. PPV for CIN3+ for ASC-US+, ASC-H+ and HPV mRNA test were 9.5%, 30.9% and 16.1% PPV for CIN3+ was 19.9% and 40.1% for a double positive co-test using cut-off ASC-US+ and ASC-H+. The risks of CIN3+ were 1.1%,1.6% and 1.4% in cytology negative with cut-off ASC-US+, ASC-H+ and HPV mRNA negative.

The risks of CIN3+ were 0.6% and 0.9% with a negative co-test using cut-off ASC-US+ and cut-off ASC-H+.

References

The 3-type HPV mRNA test has low positive rate and holds high PPV for CIN3+ in women 21-29 years. Co-testing with cut-off ASC-US+ reduced risk for CIN3+ from 1.1% in women with normal cytology to 0.6% in women with a negative co-test. The number of women to be followed up can be reduced from 24.8% using cytology alone (ASC-US+) to 12.0% by using co-test positive with cut-off ASC-H+ without affecting the low risk of CIN3+ in test negative women. Co-testing women in their 20`s with cytology and HPV mRNA improves women’s safety and reduces over referral and overtreatment. By providing clinicians an improved opportunity to address elevated risk, a more accurate patient management can be effectuated.

00111

CLINICAL VALIDATION OF THE COBAS 6800 HPV TEST FOR CERVICAL SCREENING

13. Screening methods

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Background / Objectives

We wished to evaluate the cobas 6800 HPV test in the setting of organized cervical screening, using CIN3+ as the outcome and the cobas 4800 HPV test as the comparator test.
The organized cervical screening program in the Stockholm county, Sweden uses primary HPV screening with the cobas 4800 test and stores all residual ThinPrep liquid based cytology (LBC) cervical samples at minus 25 C after clinical testing.

Results

The samples stored during 2014 and 2015 were linked to the national cervical screening registry to identify all histopathologies taken after the stored LBC sample from these women. We identified stored samples taken <6 months before diagnosis of CIN3+ from 470 women 30-64 years old (the age group targeted by HPV screening in Sweden) and with an HPV test result on file. Controls were matched 2:1 to the case women by birth year and calendar year of sampling and were required to not have a CIN3+ histopathology after the LBC sample, be negative in the HPV screening of the index sample as well as in the cytology screening in the previous screening round. Samples were retrieved from the biobank and tested with both the cobas 4800 and 6800 systems and results were compared to the original 4800 data from the same sample before storage.

Conclusion

Retrieval could be completed for 468 cases and 938 controls. In the original cobas 4800 testing, 466/468 case women were positive. In the 4800 testing of the biobanked samples 462/466 case women were positive and in the 6800 testing of the biobanked samples 462/466 case women were positive.By design, none out of 938

controls were positive in the original cobas 4800 analyses. 3/936 were positive in the 4800 analyses of biobanked samples and 8/933 were positive in the 6800 analyses of biobanked samples. Direct comparison of the 4800 and 6800 in the same archival samples showed a very high concordance (97% of samples with identical results). Variability regarding “invalid” results was found in 9 samples, 7 samples positive in 4800 were negative in 6800 and 12 samples positive in 6800 were negative in 4800.

References

In summary, the cobas 6800 had an overall sensitivity for subsequent histopathology-verified CIN3+ of 99,1%. Compared to the cobas4800 comparator test the relative sensitivity was 100% and the relative specificity was 99,5%.

FC 08. Vaccines 2

00110

LONG-TERM HUMORAL RESPONSE AGAINST NON-VACCINE ONCOGENIC TYPES HPV-31 AND HPV-45 ELICITED BY THE HPV-16/18 VACCINE IN GIRLS AGED 10-14 YEARS: 10-YEAR FOLLOW-UP DATA

05. HPV prophylactic vaccines

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Background / Objectives

Human Papilloma Virus (HPV)-16/18 AS04-adjuvanted vaccine has been shown to induce immune responses against phylogenetically-related non-vaccine types. However the durability of such cross-reactive immune responses is unknown. Here we report the 10-year humoral responses against non-vaccine type HPV-31 and -45 in girls vaccinated with HPV-16/18 AS04-adjuvanted vaccine.

Results

Girls aged 10–14 years who received 3 doses of the HPV-16/18 AS04-adjuvanted vaccine at Month 0, 1, and 6 in the initial observer-blind, randomized, controlled study (NCT00196924) and included in the immunogenicity subset were invited in follow-up studies (NCT00316706 and NCT00877877), with a total follow-up of 10 years after initial vaccination. In this post-hoc analysis, one hundred fifty subjects were randomly selected among the 418 subjects included in the according-to-protocol (ATP) cohort for immunogenicity evaluation of the cross-reactive immune response. Anti-HPV-31 and -45 antibodies were measured in serum samples collected at Month (M) 0, 7, 24, 72 and 120 by enzyme-linked immunosorbent assays (ELISA), using type-specific recombinant virus-like particles as coating antigens.

Seropositivity was defined as antibody titers ≥ 59 ELISA Unit (EU)/mL for anti-HPV-31 and -45.

Conclusion

Among the girls from the ATP cohort who were seronegative for the type analyzed before vaccination and were followed-up up to M120, all had seroconversion to anti-HPV-31 and -45 at M7; at M120, 87.7% and 85.1% remained seropositive for anti-HPV-31 and -45, respectively. The anti-HPV-31 geometric mean titer (GMT) peaked at M7 [2030.5 EU/mL; 95% CI: 1766.2, 2334.4] and decreased to 242.9 EU/mL [95% CI: 201.4, 293.0] at M120. Similarly, the anti-HPV-45 GMT peaked at M7 [2300.8 EU/mL; 95% CI: 2036.8, 2599.0] and decreased to 204.7 EU/mL [95% CI: 170.0, 246.6] at M120.

References

The HPV-16/18 AS04-adjuvanted vaccine, administered at age 10-14 years, induced humoral responses to the non-vaccine types HPV-31 and HPV-45 up to 10 years, supporting the long-term cross-protection observed for the same HPV types.

00216

EPIDEMIOLOGIC IMPACT OF A GENDER-NEUTRAL NONAVALENT HPV VACCINATION PROGRAMME IN COMPARISON TO THE CURRENT GENDER-NEUTRAL QUADRIVALENT HPV VACCINATION PROGRAMME IN SWITZERLAND

05. HPV prophylactic vaccines

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Background / Objectives

An infection with high-risk human papillomavirus (HPV) is the obligatory aetiological factor for the development of cervical cancer. In Switzerland, the prevention strategy for cervical cancer is based on primary prevention via HPV vaccination and secondary prevention with an opportunistic screening programme for precancerous lesions. Vaccination is recommended to 11-26 years old males and females. The objective of the study was to assess the epidemiological impact of switching from the currently implemented programme with the quadrivalent vaccine to the nonavalent vaccine, in an 11-26 years old gender neutral vaccination programme in Switzerland.

Results

A previously validated dynamic transmission model of HPV infections was adapted and calibrated to the Swiss setting assuming an 80% coverage rate in HPV-vaccination and lifelong vaccine type-specific protection. Only cervical disease was taken into account as statistics for other malignant and non-malignant disease caused by HPV were not available in Switzerland. The adaptation was achieved through collection and selection of the most appropriate data to reflect the current Swiss epidemiological and medical context as closely as possible. A gender neutral vaccination programme (males and females) for 11-26 years old with a nonavalent HPV vaccine was compared to the current 11-26 years old gender neutral quadrivalent vaccination programme.

Sensitivity analyses were conducted in order to test the impact of lower vaccination coverage rates and a shorter duration of protection on the model outcomes.

Conclusion

In Switzerland, the nonavalent vaccination programme would result in the additional prevention of 2,983 cervical cancer cases and 28,892 CIN 2/3 cases, compared to the quadrivalent vaccination programme over 100 years. These additional disease cases avoided would correspond to a 24% and 41% cumulative incidence decrease in cervical cancer cases and CIN 2/3 cases, respectively. It would also prevent an additional of 742 cervical cancer-related deaths over 100 years. Results of these analyses are robust since a substantial additional reduction in cervical cancer and precancerous lesions burden is still observed when varying the vaccination coverage rate from 30% to 60% or reducing the duration of protection to 20 years.

References

The switch to the nonavalent vaccine in Switzerland to prevent cervical diseases showed an important contribution in terms of public health impact compared to the quadrivalent vaccine in an 11-26 years old gender-neutral population, even with very conservative assumptions such as low coverage rates or low duration of protection and limiting analysis to only cervical diseases.

00235

OCCURRENCE OF HUMAN PAPILLOMAVIRUS (HPV) TYPE REPLACEMENT BY SEXUAL RISK-TAKING BEHAVIOUR GROUP: POST-HOC ANALYSIS OF A COMMUNITY RANDOMIZED CLINICAL TRIAL

05. HPV prophylactic vaccines

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Background / Objectives

Human papillomavirus (HPV) vaccination programs may cause an increase in non-vaccine HPV types if these eventually take over the vacated ecological niche of the vaccine types. The prerequisites and likelihood of this process known as type replacement probably are different in subpopulations with different risk of acquiring HPV infections. We examined over-time occurrence of non-vaccine HPV types among subgroups with different transmission probabilities up to 8 years post moderate coverage vaccination.

Results

We randomized 33 communities to three arms: Arm A gender-neutral HPV16/18 vaccination, Arm B girls-only HPV16/18 vaccination and hepatitis B-virus (HBV) vaccination of boys, and Arm C gender-neutral HBV vaccination. Out of 1992-94 born resident boys (31,117) and girls (30,139), 8,618 boys and 15,615 girls were vaccinated in 2007-9. Respectively, in 2010-13 8,868 HPV16/18 and non-HPV vaccinated females, and in 2014-16 5,574 initially (2007-9) or cross (2010-13) HPV16/18 vaccinated females attended two follow up visits for cervico-vaginal sampling aged 18.5 and 22 years. The samples were typed for HPV6/11/16/18/31/33/35/39/45/51/56/58/59/66 using PCR followed by MALDI-TOF MS. HPV prevalence ratios (PR) of Arms A/B to Arm C were stratified by *Chlamydia*

trachomatis status, a surrogate of risk taking behaviour. This is an ancillary study to the GSK-sponsored HPV-040 randomized trial (NCT00534638) comparing the overall protective effectiveness of gender neutral and girls-only vaccination strategies.

Conclusion

At the 1st and 2nd follow up visits the PRs in the *C. trachomatis* positives and negatives did not significantly differ for vaccine protected HPV types. Among the initially vaccinated 18.5 year-old females, the HPV52 PR was increased in the *C. trachomatis* positives only (HPV52 PRpos=2.5, PRneg=0.8), but the HPV51 occurrence was consistently somewhat increased (HPV51 PRpos=1.4, PRneg=1.4). In the initially non-HPV vaccinated 18.5 year-old females the HPV51 PR was significantly higher in the *C. trachomatis* positives than in the *C. trachomatis* negatives (HPV51 PRpos=3.8, PRneg=1.2). Among the 22 year-old females, no corresponding significant patterns were observed when comparing the initially HPV16/18 vaccinated to cross-vaccinated but initially non-HPV vaccinated females.

References

The patterns of HPV occurrence post-vaccination may differ in the core group as compared to the general population. However, no consistent over-time indications for type replacement in the vaccinated females were found in either group, although HPV51 merits further follow-up.

00272

HEALTH IMPACT AND COST EFFECTIVENESS OF IMPLEMENTING GENDER-NEUTRAL NONAVALENT VACCINATION IN FLANDERS, BELGIUM

05. HPV prophylactic vaccines

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Background / Objectives

To assess the health impact and cost-effectiveness of gender-neutral HPV vaccination (2 doses, ages 12-13) with a nonavalent HPV vaccine (9vHPV) that protects against the 9 types (6, 11, 16, 18, 31, 33, 45, 52, and 58) responsible for most HPV-related cancers and diseases compared with the current program in Flanders, Belgium, which uses 2-dose bivalent HPV vaccine (2vHPV; HPV16/18) in girls only.

Results

Population-level impact over a 100-year time horizon was simulated in both boys and girls >13 years old using a validated disease transmission dynamic model calibrated to the Flanders region to compare the 2 immunization strategies for prevention of HPV-related cervical cancer (CC); cervical lesions (CIN 2/3, CIN1); vulvar lesions (VaIN); vulvar, vaginal, penile, anal, and head and neck cancers; recurrent respiratory papillomatosis (RRP); and genital warts (GW). Relevant epidemiological, clinical, and cost data were derived from Belgian sources or international literature; GW incidence rates were obtained from a local Belgian study (Dominiak-Felden, 2015). The incremental cost-effectiveness ratio (ICER) was calculated from cost (2017 €) and quality-adjusted life years (QALYs) from this model, at discount rates of 3% and 1.5%, respectively. Deterministic sensitivity analyses were conducted.

Conclusion

Analyses suggest a gender-neutral 9vHPV vaccination program would result in cumulative reductions vs 2vHPV in HPV6/11/16/18/31/33/45/52/58-related-disease

incidence: 18.3% for CC (3,375 cases); 36.9% for CIN2/3 (16,115 cases); 41.2% for CIN1 (11,899 cases); 18.8% and 5.3% (485 and 187 cases) for male and female anal cancer, respectively; 30.1% for penile cancer (514 cases); 85.1% and 85.4% (640 and 808 cases) for male and female RRP, respectively; 84.8% and 85.4% (176,677 and 281,658 cases) for male and female GW, respectively. ICER of implementing 9vHPV gender-neutral vaccination versus 2vHPV vaccination in girls is 5,687€/QALY. Sensitivity analyses show cost effectiveness is maintained with more conservative GW estimates (6,127€ per QALY), when restricted to on-label indications (11,782€/QALY), and versus a quadrivalent HPV vaccine (8,010€/QALY).

References

A gender-neutral nonavalent vaccination program could reduce HPV-related cancers and diseases and be cost-effective compared with the current bivalent program in girls only in Flanders, Belgium.

References

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00275

END OF STUDY RESULTS OF A 2 YEAR MULTICOUNTRY PHASE IV RANDOMIZED COMPARATIVE STUDY OF IMMUNOGENICITY AND SAFETY OF THE AS04-HPV-16/18 VACCINE AND THE HPV-6/11/16/18 VACCINE IN HIV-POSITIVE FEMALE SUBJECTS AGED 15-25 YEARS

05. HPV prophylactic vaccines

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Background / Objectives

Human immunodeficiency virus (HIV) infected subjects are at higher risk of human papillomavirus (HPV) infection. We evaluated the immunogenicity and safety of GSK's AS04-adjuvanted HPV-16/18 vaccine (AS04-HPV-16/18) as compared to Merck's HPV-6/11/16/18 vaccine (4vHPV) when administered to HIV-positive (HIV+) females aged 15-25 years.

Results

In this 2-year, Phase IV, observer-blind, randomized, controlled study (NCT01031069) clinical stage 1 HIV+ subjects and HIV-negative (HIV-) subjects were vaccinated with 3 doses of either vaccine (0, week 6, month 6). Anti-HPV-16/18 antibodies were measured by pseudovirion-based neutralizing assay (PBNA) at Month 0 and 7 and by enzyme-linked immunosorbent assay (ELISA) at all timepoints. HPV-16/18 specific T-cell and B-cell mediated immune responses were assessed by intracellular cytokine assay and enzyme-linked immunospot. Safety outcomes were recorded.

Conclusion

Total vaccinated cohort (TVC) included 257 HIV+ and 289 HIV- subjects. Immunological superiority of the AS04-HPV-16/18 over 4vHPV was demonstrated in HIV+ and in HIV- subjects for HPV-16 and HPV-18 at Month 7 (TVC). At Month 7, in HIV+, anti-HPV-16 and HPV-18 PBNA titers were 2.74 and 7.44 fold higher ($p<0.0001$), respectively, in AS04-HPV-16/18 group compared to 4vHPV group. In HIV-, ratios were 3.05 and 5.38, respectively ($p<0.0001$). Non-inferiority of immune response of AS04-HPV-16/18 in HIV+ over 4vHPV in HIV- was shown in the according to protocol cohort (ATP). At Month 24, antibody concentration by ELISA in the AS04-HPV-16/18 groups remained above those in the corresponding 4vHPV groups for both antigens, and overall, antibody responses appeared lower in HIV+ versus HIV-. Seroconversion rates at Month 24 in HIV+ for HPV-16 were 100% in the AS04-HPV-16/18 and 94.7% in the 4vHPV groups, and were 96.3% and 67.6% for HPV-18, respectively (in HIV-: 100% in all except for HPV-18 in 4vHPV: 98.5%) (ATP). There was a trend for better CD4 and B-cell response with AS04-HPV-16/18 versus 4vHPV. CD4 response was similar in HIV- and HIV+. B-cells response appeared better in HIV- versus HIV+. No safety concerns were raised.

References

In this 15-25 year-old cohort of HIV+ and HIV- women, AS04-HPV-16/18 was shown immunologically superior to 4vHPV. Antibody response remained sustained over 24 months but appeared lower in HIV+ versus HIV- for both vaccines. AS04-HPV-16/18 has the potential to induce a longer-lasting protection against HPV-related lesions and cancers in HIV+ and HIV- compared to 4vHPV.

00374

BIVALENT HPV VACCINE EFFECTIVENESS CORRELATES WITH PHYLOGENETIC DISTANCE TOWARDS VACCINE TYPES 16 AND 18

05. HPV prophylactic vaccines

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Background / Objectives

We previously demonstrated cross-protection against specific oncogenic HPV types among Dutch women aged 16 to 24 years, who had been eligible for vaccination with the AS04-adjuvanted bivalent vaccine (2vHPV) since 2009 and visited sexually transmitted infection clinics in the Netherlands between 2011 and 2015 [1]. To reconcile inconsistencies in cross-protection reported across 2vHPV studies, and to substantiate the presumed type-restricted nature of protection elicited by virus-like particles (VLPs) targeting L1 capsid protein, we re-evaluated vaccine effectiveness (VE) against type-specific HPV positivity as a function of phylogenetic distance.

Results

We recalculated type-specific VE by the logistic mixed model from the original publication [1], for all genotypes in the SPF10-LiPA25 assay. Phylogenetic distance was calculated from reference DNA sequences (including those used for construction of VLPs) obtained via the PapillomaVirus Episteme database [https://pave.niaid.nih.gov/]. We performed a phylogenetic analysis based on L1 amino acid composition, as well as on *L1* capsid gene and whole genome sequences (WGS). We fitted penalized regression splines to VE as a function of minimum distance of each type to L1 VLP in protein analysis, and to HPV-16 or 18 reference sequences in DNA analyses.

Conclusion

Overall, there was a clear relationship between VE and phylogenetic distance to L1 VLP (Spearman's ρ= −0.68, p<0.001). Predictions from the estimated spline function suggest that 2vHPV provides partial cross-protection against HPV-31, 33, 35, 45, 52, and 58, i.e. high-risk types with close phylogenetic relationships to HPV-16 or 18. Cross-protection to low-risk types, including HPV-6 and 11, is not to be expected. Analyses based on *L1* capsid gene (ρ= −0.65, p<0.001) and WGS (ρ= −0.77, p<0.001) yielded comparable results as those for L1 protein. In partial correlation analysis, WGS phylogenetic distance to HPV-16 or 18 was the strongest independent determinant of VE.

References

Our findings indicate that WGS phylogenetic distance to HPV-16 or 18 better explains cross-protection by 2vHPV than distance measures based solely on L1, and suggest that cross-protection induced by 2vHPV primarily extends to the same high-risk types, albeit with moderate efficacy, as included in the nonavalent HPV vaccine.

References

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00476

Systematic literature review of neutralizing antibody immune responses to non-vaccine high-risk HPV types induced by the bivalent and the quadrivalent vaccineses

05. HPV prophylactic vaccines

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Background / Objectives

Partial and inconsistent efficacy against some HPV types not targeted by the bivalent (2v) and quadrivalent (4v) vaccines has been reported. We reviewed the literature on neutralizing antibody immune responses to non-vaccine high-risk HPV types 31, 33, 45, 52 and 58.

Results

PubMed/EMBASE were systematically searched for full-text, original articles, published in English from Feb 2008–Feb 2018, reporting serum antibody responses against non-vaccine HPV types using pseudovirion-based neutralization assays. Studies of healthy subjects receiving 3 vaccine doses were included; immunocompromised populations were excluded. Data extracted included seropositivity (% of subjects with antibody titer above a study-specific threshold) and antibody titers by time from 1st vaccine dose.

Conclusion

Seven publications met inclusion criteria: 3 reported on the 2v vaccine, and 4 (3 RCTs and 1 cohort study) compared the 2 vaccines. Among adolescent girls, seropositivity for HPV 31 at 7–12 months post 1st dose was significantly higher with the 2v than with the 4v vaccine in one (93 vs 56%, P<.05, N=50) but not in another study (97 vs 89%, P>.05, N=188); seropositivity for HPV 45 was higher with the 2v vaccine in both studies (36 vs 6%, P<.0001 and 64 vs 19%, P<.05). Seropositivity with the 2v vs the 4v vaccine was 74 vs 53% for HPV 33, 64 vs 19% for HPV 52 and 39 vs 20% for HPV 58 (N=188). Among young women, one 2v vaccine study (N= 45)

reported 74% seropositivity for HPV 31 and 61% for HPV 45 at 12 months compared to 11% and 13%, respectively, at baseline. However, an RCT (N=27) of young women found that neither of the 2 vaccines induced HPV 45-neutralizing antibodies. Among adult women (18–45 years, N=1106), seropositivity with the 2v versus the 4v vaccine for HPV 31 was 69 vs 40% at month 7, but dropped to 28 vs 29% at month 24; for HPV 45, seropositivity was 24 vs 6% at month 7, dropping to 15 vs 3% at month 24. In contrast, one small 2v vaccine study (N=12) reported that HPV 31 and 45 neutralizing antibody responses were maintained over 36 months. Vaccine-induced HPV 31 and 45 neutralizing antibody titers were generally 2–4 logs lower than titers against their related vaccine-targeted types, 16 and 18.

References

In contrast to neutralizing antibody responses to types covered by HPV vaccines, HPV 31-antibodies are detected within 6 months of the 3rd dose in some but not all females receiving the 2v or 4v vaccines. Seropositivity rates, particularly for HPV 45, are variable, and neutralizing antibody titers significantly lower than titers against vaccine-targeted types. The largest published study suggests limited durability of cross-neutralizing antibodies.

00523

TYPE-SPECIFIC DATA ON HUMAN PAPILLOMAVIRUS INFECTION IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA IN THE ASIA-PACIFIC REGION

27. HPV and oropharynx / Head and neck cancer

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Background / Objectives

To assess the availability of recent type-specific data on human papillomavirus (HPV) infection in oropharyngeal squamous cell carcinoma (OPSCC) and report type-specific HPV prevalence in OPSCC in the Asia-Pacific region.

Results

PubMed/Medline and EMBASE databases were systematically searched for full publications reporting type-specific HPV DNA detection in histologically confirmed OPSCC. Bibliographies were also searched. Original studies reporting on all of the following were included: overall HPV, types 16 and 18 and ≥1 other high-risk type. Key exclusion criteria were: publication before 2012, not English, special populations (e.g., HIV-infected only) and small study (N<25). Key information, including study type, country, population characteristics, sample type, HPV assay, HPV types detected, p16INK4a expression, and E6/E7 mRNA detection, was extracted.

Conclusion

Fourteen publications reporting on type-specific distribution were included: 13 reported data on OPSCC overall, 5 on tonsillar SCC, 4 on base of tongue SCC, and 2 on other OPSCC sites. Six studies originated from Eastern Asia (China and Japan only), 3 from Southern Asia (India only), 2 from Oceania (Australia and New Zealand), and 1 each from South-Eastern (Singapore), Central (Kazakhstan) and Western Asia (Israel). Across the Asia-Pacific region, HPV DNA was detected in 43.5% of 1,707 OPSCC cases overall, 50.0% of 142 tonsillar SCC, 20.9% of 239 base of tongue SCC, and 5.5% of 55 OPSCC cases from other anatomic sites. HPV

16 was detected in 88.4% of HPV-positive OPSCC, followed by HPV 18 (5.4%), HPV 31 (3.2%), HPV 45 (3.0%) and HPV 33 (1.3%). The prevalence of other HPV types was <1%.

References

Most of the recent data on HPV type-specific distribution in OPSCC in the Asia-Pacific region originates from Eastern Asia. Detection of HPV OPSCC varies across anatomic subsites and is most common in tonsillar SCCs. HPV 16, 18, 31, 45 and 33 are the types that were identified.

FC 09. Anal neoplasia

00278

Anal liquid-based cytology and high risk human papilloma testing as composite endpoint in HIV-infected men who have sex with men to optimize screening for anal neoplasia

09. HPV screening

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Background / Objectives

Screening methods for anal intraepithelial dysplasia (AIN) are suboptimal, require high observer experience and anal cytology has a lower specificity than cervical cytology. This study aimed to determine the diagnostic performance of a composite endpoint comprising anal liquid-based cytology (aLBC) and high-risk human papillomavirus (HR-HPV) testing to predict histological high-grade squamous intraepithelial lesions (hHSIL).

Results

From a cohort of HIV-infected men who have sex with men (MSM) seen at a Spanish University hospital, all patients who had an aLBC with concomitant HR-HPV testing were included. hHSIL were determined by high-resolution anoscopy (HRA)-guided biopsy and included AIN grade II-III.

Conclusion

A total of 705 visits obtained from 426 patients were included. The prevalence of HR-HPV among the different aLBC results were: 51.9% (133/215) normal, 87.9% (20/232) low-grade squamous intraepithelial lesions (LSIL), 13.3% atypical squamous cells of unknown significance and 90.9% (149/164) HSIL; p(linear

association)<0.001. A low prevalence of hHSIL was only observed for the composite aLBC/HR-HPV-testing endpoint “normal/noHR-HPV” (10%) and “LSIL/noHR-HPV” (4%), while 29% of those with normal cytology but HR-HPV showed hHSIL. The prognostic values (95% confidence interval) for HR-HPV to predict hHSIL in normal cytology were: Sensitivity, 83% (69.2%-92.4%); specificity 44.1% (36.4%-51.9%); positive predictive value (PPV), 29.3% (25.6%-33.3%), negative predictive value (NPV), 90.2% (82.8%-94.7%). Corresponding figures for cytologic LSIL were: Sensitivity, 98.8% (93.3%-99.9%); specificity, 17.9% (12.1%-24.9%); PPV, 39.2% (37.4%-41.1%); NPV, 96.4% (78.9%-99.5%). Here, only 4% of those without HR-HPV showed hHSIL. A positive interaction and a synergistic effect for the composite endpoint was observed (relative excess risk=1.50, attributable proportion of histological results to the interaction=0.17, synergy index=1.24). Given the high proportion of hHSIL in those patients with normal aLBC/HR-HPV despite the considerably low VPP, as well as the almost absent prevalence of histological HSIL in those without HR-HPV despite cytological mild dysplasia, it should be considered to modify the widely used recommendations to refer all patients with cytological LSIL to HRA, while sparing HRA in individuals with normal cytology.

References

HRA may be spared in the setting of LSIL/noHR-HPV followed by aLBC-based screening. In contrast, HIV-infected MSM with normal aLBC but HR-HPV infection should be considered for
HRA.

00279

Assessment of the learning curve of high-resolution anoscopy in HIV-infected men who have sex with men: how to improve the performance?

09. HPV screening

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Background / Objectives

High-resolution anoscopy (HRA) with subsequent biopsy to detect high squamous intraepithelial lesions (HSIL) is characterised by a long learning curve. Starting the learning process, especially without supervision, is difficult and based on studying a high number of images and the scarce number of manuals available, as well as revising the results with a specialized pathologist. This study aimed to determine the required learning time and to identify factors that impact on the training process.

Results

From September 2010 until July 2017, all HIV-infected men who have sex with men seen at one consultancy of a tertiary care centre in Spain, were invited to be screened for HSIL by means of HRA with biopsy. In the present study, all those who for the first time underwent HRA with subsequent anal biopsy conducted by one single observer and who had no prior test for anal lesions including digital-rectal examination, HPV testing or anal liquid-based cytology (aLBC), were included.

Conclusion

Eighty-five (14.7%) of the 581 patients included presented histological HSIL. The factors associated with the capacity to detect HSIL in biopsy were the presence of cytological HSIL [adjusted odds ratio (aOR): 3.04, 95%CI: 1.78-5.21; p<0.001], infection with high-risk human papilloma virus (HR-HPV) (aOR: 2.89, 95%CI:1.38-

6.05; p=0.005), the number of biopsies taken per HRA (aOR: 1.28, 95%CI: 1.07-1.52; p=0.006) and tobacco smoking (aOR: 1.75; 95%CI: 1.12-2.73; p=0.014). Two events independently augmented the detection rate of HSIL: first, the moment one single experienced pathologist interpreted biopsies after 409 HRA (aOR: 2.80, 95%CI: 1.74-4.48; p=0.035) and second, the HRA observer underwent an additional training after 536 HRA (aOR: 2.57, 95%CI: 1.07-6.16; p=0.035). The prevalence of histological HSIL was 9.3% until the first event, 22.8% between the two events and 39.1 after the second event. A learning process could be observed throughout the whole study period without an increase of HR-HPV prevalence.

References

The long learning process supports the growing evidence that the proposed training volume of 50-200 performances is underestimated. Extensive training of both anoscopist and pathologist is warranted and the development of tools to support the diagnostic performance may be considered.

00059

LONG-TERM PERFORMANCE OF HPV GENOTYPING, HPV E6/E7 MRNA EXPRESSION, AND P16/KI-67 CYTOLOGY FOR DETECTION OF ANAL PRECANCER IN HIV+ MSM

25. Anal neoplasia

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Background / Objectives

Biomarkers of HPV-related cervical carcinogenesis may have applications for anal cancer screening in high-risk populations such as HIV-positive (HIV+) men who have sex with men (MSM); however, prospective studies are needed to evaluate the long-term reassurance and safety of a negative test result. Here, we evaluated the longitudinal performance of several biomarkers including high-risk (HR) HPV DNA testing, HPV16/18 genotyping, HPV E6/E7 mRNA, and p16/Ki-67 dual stain (DS) in a population of HIV+ MSM.

Results

This study includes 363 HIV+ MSM enrolled at an HIV/AIDS clinic between August 2009 and June 2010 with passive follow-up through October 2015. All men had anal cytology and high-resolution anoscopy (HRA) at baseline; cytology and histology disease endpoints were combined to account for potential misclassification by HRA and biopsy placement. We calculated the sensitivity and specificity of each biomarker for anal intraepithelial neoplasia grade 2 or worse (AIN2+) detection at baseline and at the end of follow-up. For each biomarker, we estimated the cumulative risk of AIN2+ at 2 and 5 years by summing the probability of prevalent disease and incident disease risk calculated from logistic and Cox regression models, respectively.

Conclusion

Among the 363 men included in our study (median age 53 years), 167 had no dysplasia (46%), 92 had AIN1 (25%), 48 had AIN2/HSIL (13%), and 56 had AIN3/HSIL (16%) at baseline. Of 259 with <AIN2 at baseline, a total of 135 (52%) had follow-up (mean = 2.8 years) and 25 developed incident AIN2+ (10 AIN2 and 15 AIN3; mean follow-up time = 2.1 years). HR-HPV testing had the highest positivity and sensitivity, but the lowest specificity of all assays. HPV16/18 genotyping and HPV E6/E7 mRNA both had lower positivity and high specificity for AIN2+ detection, but much lower sensitivity compared with the other assays. The 2-year and 5-year cumulative risks of AIN2+ were highest in men testing positive for HPV16/18 (59.6% and 71.6%) and HPV E6/E7 mRNA (60.3% and 72.7%), followed by DS (52.0% and 63.8%) and cytology (51.6% and 61.4%), respectively. Men testing HR-HPV-negative (3.3% and 7.3%) and DS-negative (7.6% and 9.4%) had the lowest 2-year and 5-year cumulative risks of AIN2+, respectively. The 2-year risks for HR-HPV and DS negatives were lower than the baseline AIN2+ risk in men who were cytology-negative (8.0%).

References

Biomarkers evaluated for cervical cancer screening show long-term risk stratification for AIN2+. Baseline HR-HPV and DS negativity indicate low risk of AIN2+ for at least 2 years compared with anal cytology; however, the high positivity of HR-HPV in this population may limit its utility for surveillance and management of HIV+ MSM.

00105

HOST CELL DNA METHYLATION MARKERS FOR THE DETECTION OF HIGH-GRADE ANAL NEOPLASIA AND ANAL CANCER IN HIV+ MEN WHO HAVE SEX WITH MEN

25. Anal neoplasia

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Background / Objectives

High-grade anal intraepithelial neoplasia (AIN2/3; HGAIN) is highly prevalent in HIV+ men who have sex with men (MSM), but only a minority will eventually progress to cancer. Currently the cancer risk cannot be established, and therefore all HGAIN are treated, resulting in overtreatment. We assessed the potential of host cell DNA methylation markers for detecting HGAIN and anal cancer.

Results

A series of FFPE tissue samples of HIV+ men with anal cancer (n=26), AIN3 (n=24), AIN2 (n=42), AIN1 (n=22) and controls (n=34) were analyzed for DNA methylation of nine genes using quantitative methylation-specific-PCR. Univariable and LASSO logistic regression, followed by leave-one-out-cross-validation (LOOCV) were used to determine the performance for the detection of AIN3 and cancer.

Conclusion

Methylation of all genes increased significantly with increasing severity of disease ($p<2x10-6$). HGAIN revealed a heterogeneous methylation pattern, with a subset resembling cancer. Four genes (ASCL1, SST, ZIC1 and ZNF582) showed remarkable performance for AIN3 and anal cancer detection (AUC>0.85). The most potent marker, ZNF582 (AUC=0.89), detected all cancers and 54% of AIN3 at 93%

specificity. Slightly better performance (AUC=0.90) was obtained using a marker panel including five markers.

References

DNA methylation is significantly associated with anal carcinogenesis. A methylation marker panel, including ZNF582, can identify anal cancer and HGAIN with a cancer-like methylation pattern. Validation studies are warranted to verify their potential for the screening and management of HIV+ MSM at risk for anal cancer.

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00162

HPV Prevalence of Rectal and Scrotal Squamous Cell Cancers in the United States

25. Anal neoplasia

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Background / Objectives

Rectal squamous cell cancers (SCC) are morphologically similar to anal cancers and are thought to be caused by HPV. HPV was recently identified to cause some scrotal cancers. We determined the prevalence of HPV types in rectal and scrotal cancers in comparison with anal and penile cancers in the United States.

Results

The staff in three population-based cancer registries identified all or a sample of cases of invasive scrotal carcinomas, rectal SCC, and anal carcinomas newly diagnosed during 2014-15. One diagnostic tumor-containing block per case was serially sectioned for HPV detection and typing with confirmation of histology in sections preceding and following. L1 consensus PCR with type-specific hybridization was performed to identify 37 types. Overall and type-specific prevalence was determined. The preventable fractions of cancers were estimated based on the hierarchical proportion to HPV 16 and 18 oncogenic genotypes included in all vaccine formulations (HPV 16/18; 16/18) and the additional protection from the 5 new types in the 9-valent) vaccine (HPV 31/33/45/52/58: 5 types) Preliminary data were unweighted. We compared scrotal prevalence to penile prevalence from an earlier similar US study.

Conclusion

Samples from 72 anal, 41 rectal and 6 scrotal cancers were successfully genotyped. The prevalence of any HPV, HPV 16/18 and 5 additional types varied by anatomic site: Anal 96% [16/18:80.6%,5 additional: 8.3%]; Rectal 83% [16/18: 73.2%, 5 additional: 9.8%]; Scrotal 67% [16/18: 50%, 5 additional: 0%]. HPV prevalence of penile cancers (n=79) was 63% [16/18: 47.9%, 5 additional: 9.0%].

References

New estimates of HPV prevalence for rectal SCC are similar to anal carcinoma and that of scrotal carcinoma is similar to penile carcinomas. These estimates will be useful as a baseline measure to determine the future impact of vaccines on these two cancers.

00221

TOPICAL ABI-1968, AN ACYCLIC NUCLEOSIDE PHOSPHONATE PRODRUG FOR TREATMENT OF HPV-ASSOCIATED ANAL AND CERVICAL HSIL

25. Anal neoplasia

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Background / Objectives

ABI-1968 (Octadecycloxyethyl benzyl 9-[(2-phosphonomethoxy)ethyl]guanine) is an acyclic nucleoside phosphonate prodrug of PMEG-pp under development for topical treatment of anal and cervical high-grade squamous intraepithelial neoplasia (HSIL) caused by hr HPV infection. ABI-1968 has potent activity against diverse lr and hr HPV genotypes, inhibiting HPV DNA synthesis in a luciferase reporter gene assay (EC50 = 0.04 – 0.18 mM; CC50 >10mM). It has also been shown to be antiviral for productive HPV infection in 3D organotypic epithelial cultures causing DNA damage associated with induction of apoptosis in suprabasal strata (1, 2). ABI-1968 has been shown to induce DNA damage and arrest in S- and G2/M-phase and induction of apoptotic markers in HPV-transformed cells. The prodrug chemistry is designed to facilitate efficient transmembrane uptake into cells and controlled activation to PMEG-pp. We have used data from cellular uptake and metabolism studies of ABI-1968 and cidofovir (3) to model the integrated activation constants and intracellular elimination half-lives. The modeling reveals that ABI-1968 is activated 8-fold more slowly and has an extended elimination half-life of ABI-1968 relative to cidofovir. These properties should result in slower accumulation of the active drug species following topical treatment, potentially minimizing local toxicities and permitting less frequent yet effective dosing regimens.

Results

ABI-1968 topical cream has been studied in 40 healthy volunteers and more than 40 patients with either anal HSIL or cervical HSIL. In addition to tolerability of the cream, ABI-1968 activities are being demonstrated by histopathology and physician observations using colposcopy or high resolution anoscopy (HRA). Images from colposcopy or HRA have also been captured before and after treatment.

Conclusion

To date, weekly application of up to 5 doses is well tolerated at dosage strengths ranging from 0.01-1.0%. No dose limiting toxicities have been observed. Adverse events are generally mild and confined within the anogenital region. There have been no discontinuations due to adverse events. Preliminary changes in histopathology, lesion appearance and HPV status in these patients have been observed. No systemic exposure has been detected (assay LOQ = 0.2 ng/mL) and no systemic adverse events have been reported. Safety, PK and pharmacodynamic results will be further summarized.

References

Topical ABI-1968 is a potential topical treatment for HPV-associated HSIL. The preliminary pharmacodynamic observations and favorable clinical safety profile strongly support further development of ABI-1968 in both cHSIL and aHSIL.

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00248

PREVALENCE OF HPV AND ANOMALOUS ANAL CYTOLOGY IN HIGH-RISK WOMEN: A SINGLE-CENTRE STUDY

25. Anal neoplasia

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Background / Objectives

The appearance of anal intraepithelial neoplasia (AIN) and its progression to cancer is related to multiple factors. The identification of risk groups would allow an early diagnosis of the AIN, considering the inclusion of this location in the routine study (1,2).

The aim of this study is to compare the prevalence of Human Papillomavirus (HPV) and altered anal cytology in women with high-grade cervical dysplasia with respect to patients without injury or with low-grade injury. It has also been analyzed what other risk factors are significant.

Results

A prevalence study was conducted from April 2015 to March 2017. We recruited the new patients referred to the Pathology Unit of the Lower Genital Tract. Women diagnosed with CIN2+ were considered high risk; those without injury or CIN1 were considered low risk. Genotyping of HPV and anal cytology were performed. Those with abnormal anal cytology were referred to High Resolution Anoscopy. All included women completed a clinical questionnaire.

Conclusion

Of 171 patients recruited, 51 (29.8%) were diagnosed with CIN2+: there were no statistically significant differences in the prevalence of high-risk HPV (HR-HPV) (29% vs 30%, P = 0.9); nor in abnormal anal cytology (45% vs 27%, P = 0.1, OR 2.2, 95% CI 0.7-6.2) with respect to the low risk group.

The detection of cervical HR-HPV increases the risk of anal HR-HPV (OR 3.3, 95% CI 1.6-7.9, P < 0.05).

The prevalence of HR-HPV in immunocompromised patients, 14% of the population, is higher than in immunocompetent patients (20% vs 9%, OR 2.4, 95% CI 0.91-6.66, P < 0.05).

Predictive regression model that classifies 65.28% of the HR-HPV anal-positive population: immunosuppression (OR 4.9), cervical HR-HPV (OR 4.1), smoking (OR 0.2), P < 0.05; high grade dysplasia (OR 0.75); anal sex relations (OR 1.7), P > 0.05. S = 49%, E = 80%.

References

Other studies show the higher incidence of AIN and anal cancer in women with a history of invasive and in situ genital cancers (OR 1.82 to 16.4) (3,4,5). The appearance of high-grade AIN presents a latency of about 25 years in these patients (6). Based on our results, high-grade cervical dysplasia of recent diagnosis does not appear to be an independent risk factor for the detection of abnormal anal cytology and anal HR-HPV, but the state of immunosuppression is associated with anomalous results (7,8).

More evidence is needed to clarify the relationship between cervical and anal HPV infections; as well as the effect of the different shared risk factors.

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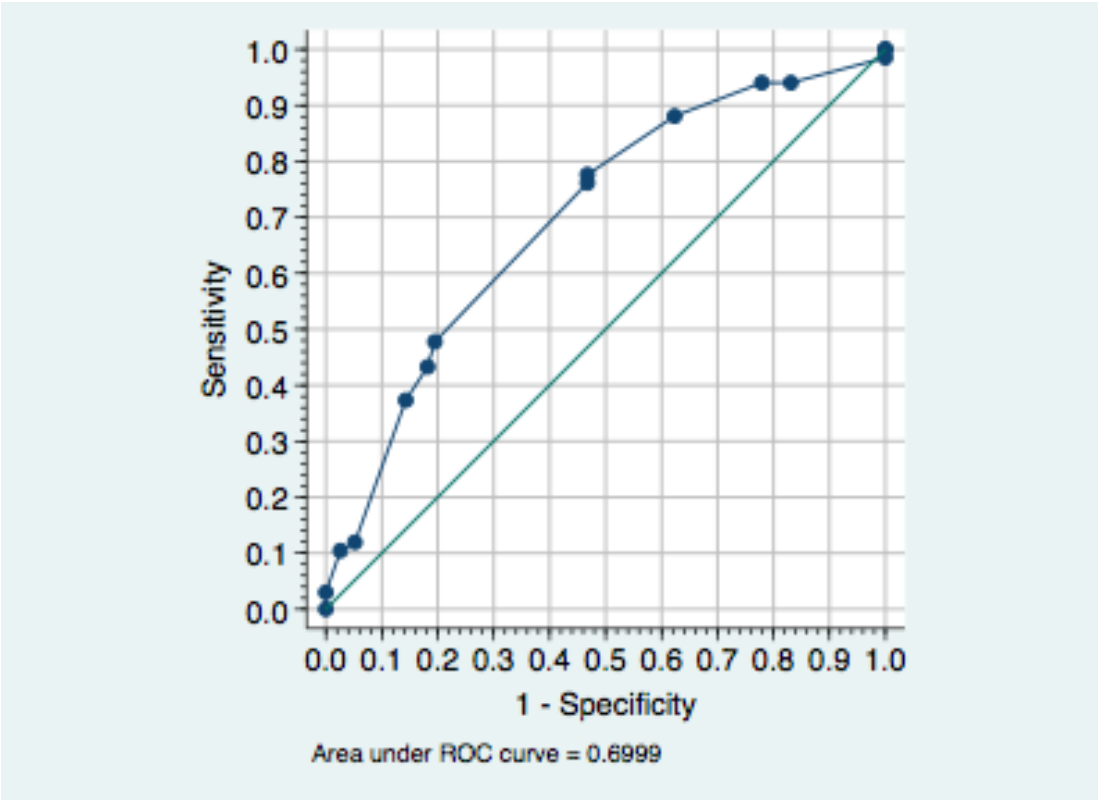

00499

SYSTEMATIC REVIEW AND META-ANALYSIS ON THE PROGNOSTIC SIGNIFICANCE OF p16INK4A AND HIGH-RISK-HPV DNA IN ANAL SQUAMOUS CELL CARCINOMA

25. Anal neoplasia

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Background / Objectives

Oncogenic human papillomavirus (HPV) types are assumed to play an etiological role in a major proportion of anal squamous cell carcinomas (ASCC). The etiological association is indicated by the detection of high-risk HPV (HR-HPV) DNA, which has been detected in up to 90% of ASCC in previous studies, and the cell cycle regulator protein $p16^{INK4A}$. By analogy to other HPV-driven tumor entities, it has been suggested that these two markers are of prognostic significance in ASCC. However, the published studies have reported heterogenous survival data stratified by these two markers and clinical variables.

Our systematic review and meta-analysis aims to determine the prognostic relevance of oncogenic HPV DNA, $p16^{INK4A}$, and clinical characteristics in ASCC.

Results

Published studies analyzing $p16^{INK4A}$ and survival in ASCC were identified by a broad search string. Authors of included studies were contacted to obtain individual patients' data (IPD). Overall survival (OS) was analyzed by Cox-Regression analyses using $p16^{INK4A}$ and HR-HPV DNA status with adjustment for relevant covariates.

Conclusion

Sixteen studies were initially identified. We received IPD from eight studies with a total of 666 patients diagnosed with an ASCC. 544 patients could be included in further analyses. 451 of a total of 538 ASCC (83.8%) overexpressed $p16^{INK4A}$ on immunohistochemistry. In 82.0% of 460 ASCC HR-HPV DNA was detected. Compared to patients with both $p16^{INK4A}$- and HR-HPV DNA-positive ASCC patients with an ASCC negative for $p16^{INK4A}$ and HR-HPV DNA had the worst OS (HR=3.3 (95% Confidence Interval (CI), 2.0-5.4), $p<0.001$) in a multi-variable analysis. Patients with discordant $p16^{INK4A}$ and HR-HPV DNA status differed regarding survival. Patients with $p16^{INK4A}$-positive, but HR-HPV DNA-negative ASCC had a worse OS than patients with a $p16^{INK4A}$-negative, but HR-HPV DNA-positive ASCC (HR=2.9 (95% CI, 1.6-5.3), $p<0.001$ and HR=2.6 (95% CI, 1.3-5.1), $p=0.005$, respectively) compared to patients with HR-HPV DNA- and $p16^{INK4A}$-positive ASCC.

References

Our systematic review and meta-analysis demonstrates that simultaneous HR-HPV DNA detection and $p16^{INK4A}$ overexpression are found in the majority of ASCC and predict a better OS. However, a combination of markers is necessary to reliably assess the prognosis of affected patients.

00307

ANAL AND ORAL HUMAN PAPILLOMAVIRUS INFECTIONS IN THE NEW ERA OF HIV-PrEP'S USERS

30. Sexually transmitted diseases and HIV infection

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Background / Objectives

Human Papillomavirus (HPV) infection is the first sexual transmitted infection (STI) worldwide and plays a major role in the development of cervical, anal and oropharyngeal cancers. One of the main risk factors described for pathogenicity is the local persistence of high-risk HPV types (hrHPVs).

Results

We conducted a prospective study single-center between May 2017 and August 2018 to assess prevalence, persistence and type of anal and oral HPV infections in a cohort of men who have sex with men (MSM) on HIV pre-exposure prophylaxis (PrEP).

Patients characteristics, anal and oral swabs (UTMTM Copan) were collected at first medical consultation (D0) and six months later (M6). Extracted DNA was amplified with AnyplexII HPV28 kit (Seegene®) allowing the detection of 19 hrHPVs and 9 low-risk HPV types (lrHPVs). GraphPad software was used to perform Spearman rank and Fisher test.

Conclusion

Fifty-eight participants were enrolled and median [IQR] age was 36 [18-74] years. Median number of different partners was 5 [1-35] per month with around 40% of anal intercourse condom-free and 18 (31%) participants used oral drugs during sex. None had been HPV vaccinated before starting PrEP. Thirty (52%) participants had previous STIs, 12 (21%) a history of condyloma and 12 (21%) at least one STI the day of HPV sampling. At D0, at least one HPV type was detected in 53 (91%) anal samples but only in 2 (3.4%) oral samples. A median of 3 [0-8] different HPV per sample was detected from anal swabs; the most prevalent hrHPVs were HPV59 (26%), HPV51 (17%) and HPV16-68-73 (each of them at 16%) and the most prevalent lrHPVs were HPV6 (26%) and HPV42 (26%). Overall, hrHPVs were found in 48 (83%) participants with a median of 2 [0-6] different hrHPVs per sample. Among them, 27 (56%) participants had at least one hrHPV covered by the 9-valent HPV vaccine. The number of hrHPVs was weakly correlated with the PrEP use duration (r_s=0.32 p=0.013) and history of STIs was a risk factor for hrHPV infection (Odd ratio=6.44, 95% CI 1.23-31.79, p=0.032). Among the 12 participants tested at M6, persistence of at least one hrHPV occurred in 9 (75%) cases. The number of different partners and hrHPV persistence were positively correlated (r_s=0.604 p=0.037).

References

This study shows a high prevalence of hrHPVs in anal samples from PrEP users, associated with the duration of PrEP and previous STIs. Although HPV infection persistence has been assessed on few patients, regular proctologic examination should be offered in order to detect associated lesions. The benefit of HPV vaccination before starting PrEP might be discussed.

FC 10. Diagnostics & management 1

00184

High correlation between clearance of High-Risk HPV strains after LLETZ and absence of residual disease in patients with early stage cervical cance

08. HPV testing

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Background / Objectives

: The standard treatment for early-stage cervical cancer is radical hysterectomy and pelvic or para-aortic lymphadenectomy. We examined whether, in patients with cervical cancer stage I A 1-I B 1, positive for High-Risk HPV (HR-HPV), clearance of the viral DNA after large loop excision of the transformation zone (LLETZ) has a high correlation with absence of cervical cancer at the final pathological specimen.

Results

Data was collected about 54 patients diagnosed with invasive cervical cancer (stage IA1- IB 1) and positive HR-HPV DNA. Shortly after the LLETZ a repeat HPV-HR test was done, before the final surgical treatment. We compared characteristics of patients with negative or positive HR-HPV from the cervix, and investigated the association of post-LLETZ HR-HPV status with residual cancer on final pathology.

Conclusion

Of 54 patients, 20 were HR-HPV negative post-LLETZ; 16(80%) had normal histology on the final pathological sample, 2 (10%) had CIN 3, and only 2(10%) had residual cancer in the final pathological specimen

Of the 34 women who were positive to HR-HPV, 8 (23.5%) were sent to chemo-radiation .The other were operated and final histological result was invasive cancer in 14 (41.2%), CIN 3 or AIS in 8(23.5%) and normal histology in 4(11.8%) women.

References

: Clearance from the cervix of HR-HPV post-LLETZ has a high correlation with the absence of residual cancer in the final surgical specimen. More studies are needed to prove if negative HR-HPV after LLETZ might serve as a new parameter for risk assessment and for less aggressive surgery in women with early stage cervical cancer.

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00532

ROLE OF DNA HPV TEST IN THE FOLLOW-UP OF WOMEN UNDERGOING EXCISIONAL SURGICAL PROCEDURES OF CERVICAL CANCER PRECURSOR LESIONS

08. HPV testing

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Background / Objectives

The evaluation of the best methods for follow treatment and a broad knowledge of the risk factors associated with greater chance of relapse are critical to achieving the best rates of successful treatment of cervical cancer precursor lesions and avoid more invasive procedures. OBJECTIVE:Evaluate the role of HPV DNA screening in the follow-up of patients submitted to excisional surgical procedures for the treatment of cervical cancer precursor lesions in the Lower Genital Tract Pathology Service of the Gynecological Clinic Division of the Hospital das Clínicas, Faculdade de Medicina da University of Sao Paulo

Results

Retrospective Cohort

Conclusion

About 85% of the patients who do this control have negative results. Other data are analyzed.

References

The possibility of post-treatment control of precursor lesions of cervical cancer with fewer and shorter exams is proving to be an excellent method for life control and the motility of this alteration. Several countries have already instituted this monitoring method with proven good results.Here we present the experience of a public hospital with limited resources in a population with low socioeconomic status

00040

JUSTIFYING CONSERVATIVE MANAGEMENT OF CIN2 IN WOMEN

20. Diagnostic procedures / management

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Background / Objectives

In 2012 the guideline from the Society of Obstetricians and Gynaecologists of Canada and the Society of Canadian Colposcopists (SOGC/SCC) changed from immediate treatment to a conservative management of CIN2 in young women [1,2]. In this study, the outcomes before and after this guideline change were reviewed in Nova Scotia, Canada.

Results

A retrospective population-based cohort study was performed among women younger than 25 years with Cervical Intraepithelial Neoplasia (CIN) grade 2, who were referred to colposcopy clinics in Nova Scotia between 2010-2014. Regression, persistence and progression rates were compared pre- and post-guideline changes.

Conclusion

Of the 636 women included in the study, 286 women were diagnosed with CIN2 before and 350 women after the guideline was changed. Women in the post-guideline period had significant more chance of a conservative approach (78.6% versus 44.1%; $p<0.001$), whereas 73.4% of the women in the pre-guideline period underwent treatment during follow-up compared to 38.9% in the post-guideline group ($p<0.001$). Regression occurred in 73.1% of all women, but women seen in the post-guideline period had a higher regression rate and lower progression rate ($p<0.05$). Histologic results from treatment specimen did not show a significant difference in

low-grade or high-grade lesions before or after the guideline has been changed (p0.59).

References

Conservative management seems safe and thus a justified approach for women younger than 25 years with CIN2.

References

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00133

OUTCOMES OF CONSERVATIVE MANAGEMENT IN WOMEN WITH TRANSFORMATION ZONE EXCISION (TZE) SPECIMENS WITH POSITIVE MARGINS

20. Diagnostic procedures / management

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Background / Objectives

In the past, appropriate management of positive margins after TZE was considered to be re-excision or even hysterectomy. Most recent recommendations advocate close surveillance in selected cases.

Our objective was to evaluate the outcomes of a case series of women with positive margins after TZE, managed conservatively, 24 months after the treatment.

Results

We performed a retrospective cohort analysis of all the cases of TZE with positive margins (CIN2+), between 2011 and 2018. We analysed the results of Pap tests, HPV tests (cobas®), colposcopies and biopsies, when performed. We considered “cured” the women with a Pap test ≤ASC-US, negative HPV test, normal colposcopy or biopsies ≤LSIL at 24 months. Evaluation at 6, 12 and 18 months was also performed.

Conclusion

Out of 201 cases of HSIL in the TZE specimen, 28 had positive margins (13.9%). We excluded 12 cases: 7 for invasive cancer, 1 had a total hysterectomy due to genital prolapse, 2 were lost to follow-up and 3 that have less than 24 months of follow-up. A total of 6 cases (60%) were considered cured, re-excision of transformation zone was performed in 5 (33%), and one (7%) is still under surveillance (positive HPV test).

The women considered cured at 24 months of follow-up were younger (35,7±5.77 vs. 41.6±11.57 years, p=0.22). On the other hand, 20% of the cases that required re-excision were post menopausal (p=0.16).

There were no differences according to the technique used (electrosurgical loop or needle excision). All cases submitted to re-excision of the transformation zone (TZ) had endocervical involvement(100% vs.66,7%, p=0.19).

A positive HPV test at 6 or 12 months was associated with the need of performing a re-excision of the TZ (33% vs. 100%, p=0.035 and 50% vs. 100%, p=0.035, respectively).

Moreover, a Pap test worse than ASC-US at 6 or 12 months was also associated with re-excision of the transformation zone (75.0% vs. 16.7%, p=0.07 and 80%vs. 11.1% of the cured, p=0.01, respectively).

References

In our series, we observed that 60% of the cases of TZE specimens with positive margins were disease free at 24 months. The need for further treatment was higher in older women, and in those with a positive HPV test and/or a Pap test > ASC-US at 6 and 12 months.

00267

IS HIGH RISK HUMAN PAPILLOMAVIRUS (HR-HPV) TESTING RELIABLE FOR THE FOLLOW UP OF WOMEN TREATED FOR GLANDULAR NEOPLASIA AND MICRO-INVASIVE CANCER

20. Diagnostic procedures / management

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Background / Objectives

The high sensitivity of Hr-HPV testing has led to its use in post-treatment follow up protocols as a “Test of Cure” (ToC) following treatment of CIN. This practice has reduced the intensity and extent of follow up, permitting a more rapid return to routine recall for the majority of women. Women treated for micro-invasive cancer (MIV) and glandular lesions generally have intense follow up and in the latter group, a greater potential of an inadequate smear due to absent endocervical material. There are little data on whether Hr-HPV testing as a ToC would be of value in these groups.

The objective was to determine utility of HPV testing as a ToC following treatment for cervical glandular lesions and MIV

Results

In Scotland, standard care for women treated for glandular neoplasia and MIV is to return to colposcopy at 6 and 12 m with smears, followed by 4 annual community smears. For this project, eligible women were those who had been treated for microinvasive squamous or adenocarcinoma, glandular abnormality (CGIN) or stratified mucinous intraepithelial lesion (SMILe). Hr-HPV testing was performed on the smears taken at 6 and 12 m post-treatment although standard care was not influenced in this observational project. Recruitment was from 2014-2016. As the main outcome was presence/absence of disease within 3 years, we present an interim analysis on those women for whom we have follow up for at least 2.5 years.

Conclusion

A total of 667 individuals were included in the cohort; 175 had at least 2.5 years follow-up from the 6 month post treatment Hr-HPV test. A total of 38/175 were treated for MIV (5 adeno; 33 squamous) and the remainder for pre-invasive glandular

lesions. Hr-HPV positivity at 6 m post-treatment was 23.6% and 14.6% in women treated for MIV and glandular lesions respectively. During follow up 7 high grade lesions were confirmed, 6 following treatment for pre-invasive glandular lesions and for 1 for MIV. Overall, specificity and PPV of a Hr-HPV test at 6 m post treatment for MIV were 78.3% (61.3%-89.6%) and 11.1% (5.8-49.3%). For preinvasive glandular lesions these values were 87.8% (80.1%-92.6%) and 20.0 (6.6%-44.2%). Sensitivity and NPV of Hr-HPV testing for MIV were 100% (5-100%) and 100% (85.4-100%) while for glandular lesions were 66.6% (24.1%-94.0%), and 98.3 (93.3%-99.7%).

References

This is an interim analysis and we await 3 year outcomes on the complete cohort. However, initial results suggest that the performance of HPV testing as a ToC after treatment for glandular abnormalities might lack the level of clinical sensitivity observed during the follow up of squamous lesions.

00147

Colposcopy Evaluation at the Time of LEEP May Avoid Unnecessary Treatment

21. Colposcopy

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Background / Objectives

The aim of the study was to assess the accuracy of colposcopy evaluation at the time of the loop electrosurgical excision procedure (LEEP) to identify women with a previous confirmatory diagnosis of squamous intraepithelial lesion/cervical intraepithelial neoplasia (SIL/CIN) with low probability of dysplasia in the LEEP specimen.

Results

We prospectively recruited a cohort of 162 women undergoing LEEP for histological high-grade SIL/CIN 2–3 or lowgrade SIL/CIN 1 with high-grade SIL cytology showing a fully visible squamocolumnar junction in the colposcopy evaluation at the time of LEEP. At the referral visit cervical cytology, human papillomavirus and genotype detection, digital colposcopy, colposcopical lesion measurement, and 1 or more biopsies of the transformation zonewere obtained. The uterine cervix was colposcopically evaluated intraoperatively.

Conclusion

Thirty-four women (21.0%) had a normal colposcopy evaluation at the time of the LEEP,whereas the remaining 128women showed abnormal findings. Absence of SIL/CIN in the LEEP specimen was confirmed in 28 (82.3%) of the 34 women with a normal colposcopy at the time of LEEP group and 8 (3.1%) of the 128 women showing abnormal colposcopy at the time of LEEP group ($p < .001$). A normal colposcopic evaluation at the time of LEEPwas associatedwith an increase in the risk of absence of lesion in the cone specimen compared with cases presenting an abnormal colposcopy (95% CI = 33.8–1,555.1, $p < .001$). The colposcopy evaluation at the time of LEEP had a positive predictive value of 82.3% (95% CI = 66.5–91.5)

and a negative predictive value of 96.9% (95% CI = 92.2–98.8) to predict low probability of SIL/CIN in the specimen.

References

Colposcopic evaluation at the time of LEEP seems to be accurate to identify SIL/CIN postbiopsy regression; thus, its performance would be considered at the time of the treatment

References

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00200

COLPOSCOPIC AND HISTOPATHOLOGIC EVALUATION OF WOMEN AGED 56-64 WITH HPV-PERSISTENCE 1 AND 3 YEARS, RESPECTIVELY, FROM THE ORGANIZED PRIMARY HPV SCREENING IN SWEDEN.

21. Colposcopy

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Background / Objectives

The aim of this study was to evaluate the colposcopic and histopathologic findings in HPV++ women age 56-64 years in an organized primary HPV screening program.

Results

Starting in 2012, the organized screening program in Stockholm randomised all resident women 56-64 years to either primary HPV screening with cytology triage or to primary cytology and HPV triage. In the HPV arm, HPV positive/cytology negative (HPV+/Cyt-) women had a repeat HPV test 1 or 3 years later. All women with HPV persistence were referred to colposcopy performed by the same expert colposcopist.

Conclusion

Among 82 women who underwent colposcopy after 1 year, 66% (54/82) had a type-specific HPV persistence and 20% were persistent for HPV 16. 42% (34/82) had a transformation zone (TZ) type 3, 51% (42/82) had atrophy, 12/82 (15%) had an abnormal cytology from the endocervix and 22% (18/82) had a HSIL in histopathology. Among 45 women who underwent colposcopy after 3 years, 58% (8/45) had a type-specific HPV persistence and 18% were persistent for HPV 16. 58% (26/45) had a TZ type 3, 64% (29/45) had atrophy, 19/45 had an abnormal cytology from the endocervix, and 7/45 had a HSIL in histopathology.

References

Colposcopic and histopathologic findings were similar for women after 1 year and 3 years of HPV persistence. TZ type 3 and atrophy is a challenge and blind as well as diagnostic cone biopsies, HPV genotyping and cytology triage are options for follow up of this group.

00402

COLPOCONNECT: USER-CENTERED DEVELOPMENT FOR A HEALTHCARE APP TO DECREASE BARRIERS TO COLPOSCOPY ATTENDANCE IN A RURAL CANADIAN SETTING

21. Colposcopy

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Background / Objectives

Numerous healthcare applications are developed each year with only a small proportion found useful to the intended users. The ColpoConnect App prototype was developed to address barriers in accessing colposcopy in northern Canada after referral for abnormal cervical cytology, and to provide a direct link for women to healthcare providers (HCP). Significant human resource and geographical challenges contribute to increased morbidity from cervical dysplasia in this underserviced population. The study objective was to collect insight from intended users to support app design incorporating known barriers to care in our rural population.

Results

A survey of women attending colposcopy (n=44) and a retrospective chart review (n=309) were done, reviewing access to colposcopy, access to technology, and barriers to attendance. The app prototype was developed using this data and employing user-centered principles through a “Hacking Health” tech start-up/population health collaboration. Low health literacy and low bandwidth functionality were optimized based on user needs. To advance app development, women were then recruited for semi-structured interviews at the time of colposcopy (n=7). Interview questions examined: hypothetical scenario with abnormal pap result,

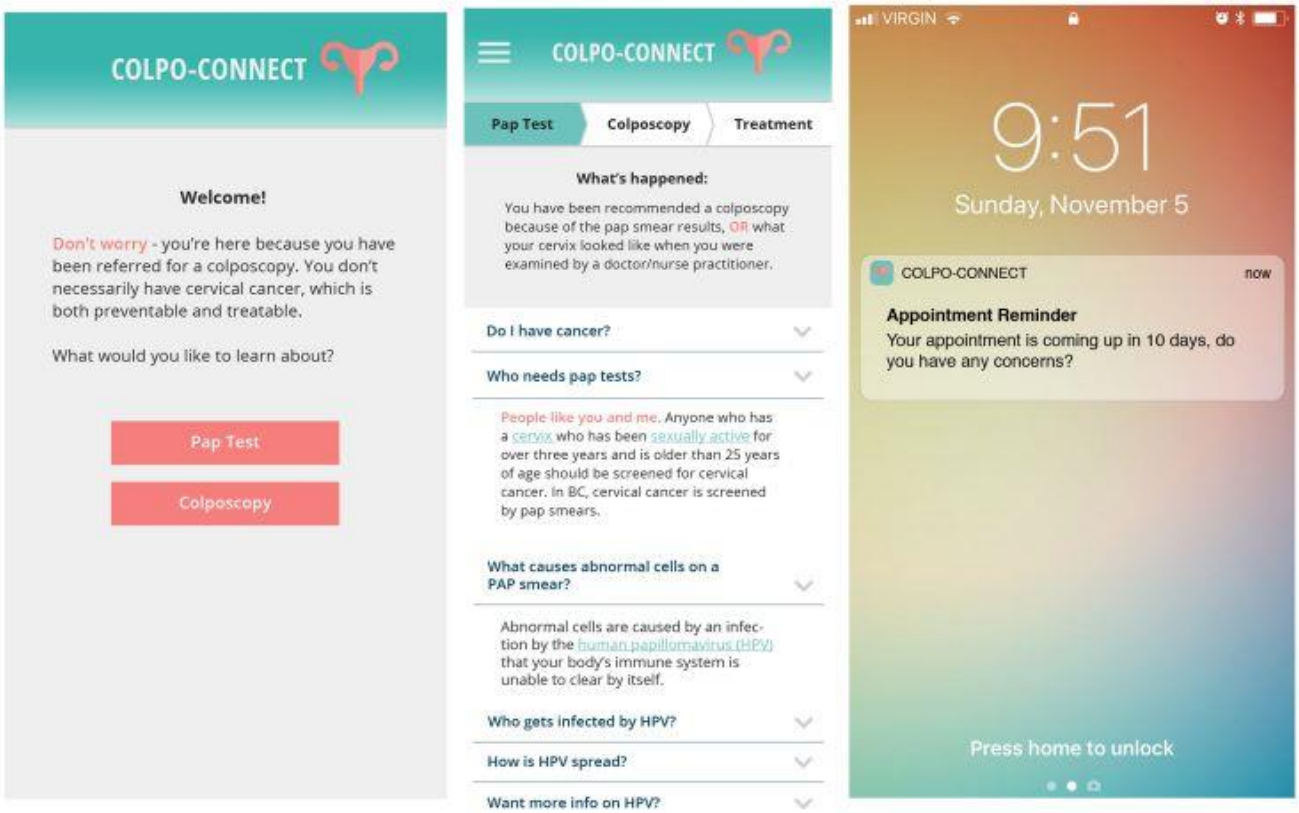
health communication, internet usage, health literacy, and initial impressions of the app prototype. Themes and sub-themes were identified from interview transcripts using Nvivo software.

Conclusion

The chart review found 25% of women referred for colposcopy did not attend their first appointment, while the patient survey showed over 77% indicating significant anxiety about their appointment, and 23% reporting no or very little knowledge of colposcopy. Further, 61% wanted to access more information and to directly connect with HCPs through text messages and phone calls. User impressions of the app were positive regarding ease of use and the inclusion of appointment reminders and themes around addressing lack of knowledge and anxiety were addressed.

References

Patient anxiety and limited knowledge are major barriers to colposcopy attendance. The ColpoConnect App aims to address anxiety by providing health information and individualized support. Providing an interface between HCPs and women referred for colposcopy is critically important in underserviced rural areas; and is crucial to improving colposcopy attendance in rural Canada with potential applications across diverse urban jurisdictions that may also be underserviced.


COLPO-CONNECT
Welcome!
Don't worry - you're here because you have been referred for a colposcopy. You don't necessarily have cervical cancer, which is both preventable and treatable.
What would you like to learn about?
Pap Test
Colposcopy
COLPO-CONNECT
Pap Test
Colposcopy
Treatment
What's happened:
You have been recommended a colposcopy because of the pap smear results, OR what your cervix looked like when you were examined by a doctor/nurse practitioner.
Do I have cancer?
Who needs pap tests?
People like you and me. Anyone who has a cervix who has been sexually active for over three years and is older than 25 years of age should be screened for cervical cancer. In BC, cervical cancer is screened by pap smears.
What causes abnormal cells on a PAP smear?
Abnormal cells are caused by an infection by the human papillomavirus (HPV) that your body's immune system is unable to clear by itself.
Who gets infected by HPV?
How is HPV spread?
Want more info on HPV?
VIRGIN
9:51
Sunday, November 5
COLPO-CONNECT
now
Appointment Reminder
Your appointment is coming up in 10 days, do you have any concerns?
Press home to unlock

00406

TEST OF CURE AFTER LEEP FOR CERVICAL INTRAEPITHELIAL NEOPLASIA

21. Colposcopy

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Background / Objectives

The most common treatment modality nowadays for cervical intraepithelial neoplasia (CIN) is loop electrosurgical excision procedure (LEEP). The test of cure after LEEP in Finland has the last decades been a follow-up colposcopy with a Pap-smear and the last years also a hrHPV-test 6 months after the procedure. The aim of our study was to compare colposcopy, cytology and highrisk-HPV (hrHPV) as a test of cure after LEEP.

Results

The study was conducted as a part of a large prospective cohort study. Patients were recruited at the Helsinki university hospital colposcopy unit during 2014-2016. All patients who had a LEEP procedure (n=462) were included. Patients were followed-up at six months by colposcopy, hrHPV-test, pap-smear and biopsies when needed.

Conclusion

Preliminary results

16.5% of the patients (n=76) were hrHPV positive at six months follow-up. Of these HPV positive patients 15.8 % (n=12) had CIN1+ and 3,9 % (n=3) had HSIL (CIN2+) in colposcopy guided biopsies. Majority of the patients (83.5 %, n=386) were hrHPV negative at six months. Of these (96.1 %, n=371) had also a negative histology. Of the hrHPV negative patients only 3,9 % had a mild histological finding of (CIN1, VIN1 or VAIN1) negative predictive value (NPV) 0.96 (95% CI 0.94-0.98) but none had HSIL histology at colposcopy NPV 1 (95% CI.0.99-1).

References

Colposcopy as a test of cure is time and resource consuming. Our findings suggest that performing colposcopy for only hrHPV positive patients could be an alternative approach and using hrHPV testing together with papsmear at 6 months provides a reliable test of cure. A longer follow-up period is needed to evaluate the natural history of hrHPV infection after LEEP.

00498

THE ROLE OF SWEDE SCORE AND MODIFIED REID COLPOSCOPIC INDEX IN THE PREDICTION OF CIN3+ LESIONS

21. Colposcopy

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Background / Objectives

Colposcopic scoring indices provide an objective tool in the diagnosis of cervical pathologies. The basic systems include Reid's colposcopic index with its variants and the Swede score and the latest modality. The aim of our study was to evaluate the significance of these indices in the prediction of CIN3+ lesions.

Results

Between years 2015-2017 we evaluated 386 patients, who underwent the colposcopic examination due to the suspicious cytological finding (ASCUS – 77 (19.9 %), LSIL – 182 (47.1 %), ASC-H - 39 (10.1 %), HSIL - 71 (18.4 %) a AGC – 17 (4.4 %)). HPV status was verified by the HC2 (Hybrid Capture® 2 test) and the colposcopic finding was scored by colposcopic indices for every patient included in our study. Subsequently we analysed the sensitivity, specificity and positive and negative predictive value of the indices for the detection of CIN3+ lesions compared to HPV assay. We excluded the patients after the surgical procedure on the uterine cervix in the past and also pregnant women from the statistical analysis.

Conclusion

Modified Reid colposcopic index with the cut-off value ≥4 showed the sensitivity 86.67 % (95% CI: 77.9 – 92.9) and specificity 81.08 % (95% CI: 76.1-85.4) for the detection of CIN3+ lesions. Swede score at the cut off value ≥ 6 achieved comparable parameters for CIN3+ lesions: sensitivity 88.65% (95% CI: 82.2 - 93.4), specificity 89.39% (95% CI: 84.8 - 92.9). Although the sensitivity of HPV DNA test showed better results compared to colposcopic diagnostics in CIN3+ lesions : 96.1%

(95% CI 89.0-99.2), the specificity was very low: 30.0% (95% CI: 23.6 - 37.1). There were no statistically significant differences between Swede score and Reid index in CIN3+ detection.

References

Swede score as a relatively new colposcopic scoring system showed in our study the best combined sensitivity and specificity in the detection of CIN3+ lesions. Both scoring systems, however, confirmed the high efficiency in the differential diagnosis of cervical pathologies. Combination of colposcopy and molecular biology methods together with the HPV DNA testing could further increase the accuracy of non-invasive screening of cervical dysplasia.

00524

The role of colposcopy at twelve months after excision of the transformation zone

21. Colposcopy

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Background / Objectives

International and national guidelines regarding the follow-up of women after LLETZ (large loop excision of the transformation zone), recommends colposcopy and cytology at 6 months. At 12 months, cytology and HPV testing are recommended and then yearly, until two consecutive negative results. However, colposcopy at 12 months remains common practice. The aim of this study is to: i) analyze outcomes from LLETZ procedures carried out for higher-grade cytology (atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion [ASC-H] or high-grade squamous intraepithelial lesion [HSIL]) associated with high-grade intraepithelial neoplasia (CIN2+) or with abnormal colposcopy findings; and ii) to assess the role of colposcopy in the management of women at 12 months.

Results

A retrospective analysis was performed of 110 women who had undergone a LLETZ procedure between January 2015 and December 2016. Demographic variables; pre-LLETZ citology and cervical biopsy results; histological results of LLETZ, maximum depth of tissue obtained and margins status; citology, cervical histology, colposcopy findings and HR-HPV test result, taken in the 2 years after LLETZ; were collected.

Conclusion

One hundred and ten LLETZ procedures were carried out. Exclusions included LLETZ for reasons other than higher-grade cytology ± CIN2+ and women diagnosed with cancer. The median age was 37,8 years with 8,8% aged over 50 years. 16,1% were nulliparous and 8,9% postmenopausal. 22,5% of women were current smokers and 60,8% were using hormonal contraception (42,2% oral pill). HPV vaccination pre-LLETZ was positive for 5,9%. Prior to LLETZ 77,4% of women had higher-grade cytology and 85,3% had CIN2+ on targeted cervical biopsy. LLETZ confirmed 80,4%

high-grade dysplasia detected on citology. Complete excision was documented for 73,7% of cases with a mean depth of 9,1mm. Regarding incomplete excision, 81,5% had an endocervical margin with dysplasia. 96,1% of patients attended their first follow-up (6 months): 12,2% had a aceto-white epithelium (AWE) and in 18,4% of colposcopies squamocolumnar junction (SCJ) was not visualized (TZ3). 20,4% of women had an abnormal cytology (≥ASC-US) at 6months and 11,3% at 12 months. 95,1% attended the 12 month visit: 21,6% of colposcopies classified SCJ as TZ3 and AWE in 14,4%. Most women had a HR-HPV test done with a positive rate of 16,5% (HPV 31 – 37,5%; HPV 16 – 31,2%). The sensitivity of colposcopy at 12 months was 0,5 and the specificity was 0,95.

References

Colposcopy after LLETZ is an examination with low sensitivity. It increases false-positive rates for high-grade lesions, potentially exposing women to anxiety and futher procedures. The high rate of SCJ not visualized after LLETZ, reduces even more the role of colposcopy at twelve months after LLETZ.

00123

HSIL in pregnancy –Observation or LLETZ in the first 15 weeks The safety of LLETZ in the first 15 weeks of pregnancy

22. Cervical neoplasia

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Background / Objectives

Large Loop Excision of The Transformation Zone (LLETZ) is the recommended treatment in women diagnosed with CIN 2 or CIN 2-3 lesions. During pregnancy observation is recommended because of the belief that during pregnancy there is no progression to malignancy and the treatment is associated with severe complications. Summarizing data from literature pregnant women over the age of 25 years with CIN 2-3 lesions has a risk of 7.4% to be diagnosed with invasive cervical cancer after delivery.

We aimed to describe the Israeli experience in pregnant women diagnosed with CIN 2 or CIN 2-3.

Results

This was a multi-center trial in which we collected data of 140 pregnant women who were diagnosed with CIN 2 or CIN 2-3 between January 2006 and May 2018.

Conclusion

Of the 27 women with CIN 2 who were observed, CIN 2-3 was diagnosed in 25%. Of the 113 women with CIN 2-3 , 63 women were followed and 50 underwent LLETZ during pregnancy. In 63 women who were evaluated after delivery the final pathological results was as follow: 4 (6.4%) were diagnosed with cervical cancer, 43 (68.2%) had CIN 2-3, 16 (25.4%) had CIN 1 or normal histology.

Of the 50 women who underwent LLETZ during the 15 weeks of pregnancy invasive cancer was diagnosed in 3(6%), CIN 2-3 or AIS in 44 women (89%) and 3 patients (6%) had CIN 1 or normal histology.

Forty three women continued their pregnancy, 39 (90.7%) of them had term deliveries, two (4.6%) had late premature deliveries (34, 36 weeks) and two women (4.6%) had missed abortion after the LLETZ.

References

The risk of cervical cancer is 6.2 % in pregnant women with CIN 2-3 diagnosed during pregnancy.

The LLETZ procedure during the first 15 weeks of pregnancy is safe. Complications included: severe bleeding, abortion, and late premature delivery in low rates , similar to the general population.

We suggest reconsidering the indications regarding CIN2-3 treatment during pregnancy in patients older than 25 years old, and consider performing LLETZ more liberally during the first trimester as it has been shown to carry minimal risks and significant benefits.

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00288

INCIDENCE OF CERVICAL CANCER AND OTHER CANCERS AFTER TREATMENT OF CIN: A SYSTEMATIC REVIEW AND META-ANALYSIS

22. Cervical neoplasia

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Background / Objectives

An increasing body of retrospective observational studies and meta-analyses suggests that CIN treatment, particularly excision, adversely affects future reproduction and the risk of prematurity(1-6). The frequency and severity of the observed adverse events is higher for the more radical techniques and with increasing cone length(1,5,7-12). This knowledge together with the ease of execution in more recent techniques like LLETZ have led to a progressive reduction in the radicality and depth of treatment.

Although all treatment techniques are highly effective in preventing recurrent pre-cancerous disease(13), several studies have documented an increase in the

incidence of cervical cancer after CIN treatment for up to 20 years post treatment. Some authors raise concerns that the progressive reduction in the radicality of treatment has led to this increased risk of future of invasion(14,15), while others advocate the move to less radical techniques for the prevention of treatment-associated perinatal morbidity and mortality(5).

Results

Design:Systematic review and meta-analysis.

Data Sources:MEDLINE, EMBASE and CENTRAL.

Eligibility Criteria:Studies with centralised follow-up assessing invasive cervical or other cancer incidence or mortality after treatment of CIN.

Data Extraction and synthesis:Pooled effect estimates for relative and absolute incidence rates were estimated using the inverse variance random-effects model with the Paule-Mandel method and between-study heterogeneity was measured using the Cochran's Q and I^2 statistics. The raw absolute incidence estimates were calculated using the variance-stabilising Freeman-Tukey double arcsine transformation. Risk of bias assessment was performed using the Quality in Prognosis Studies (QUIPS)-tool.

Main outcomes:Relative and absolute invasive cervical cancer incidence; relative incidence of other HPV-related genital tract cancer (vagina, vulva and anus), total cancer and mortality.

References

Women treated for CIN have increased incidence of not only cervical, but also of other HPV-related female genital tract cancers compared to the general population, for over 20 years after treatment. The risk is highest amongst women over the age of 50. Mortality from cervical and vaginal cancer combined is also elevated. A prolonged follow-up after the end of organised screening may be warranted for these women.

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FC 11. Vaccines 3

00046

HPV vaccine for women undergoing excisional treatment for HSIL/CIN2-3: role in the reduction of the risk of persistent/recurrent intraepitelial lesions

05. HPV prophylactic vaccines

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Background / Objectives

Up to 25% of the women treated for high-grade cervical intraepithelial lesion (HSIL/CIN2-3) present persistent/recurrent disease. Recent studies have shown preliminary evidence that a high title of antibodies against HPV could decrease the risk of recurrence in patients treated for HPV-related lesions. We aimed to provide insight into the role of HPV vaccination of women undergoing treatment for HSIL/CIN 2-3 to decrease the risk of persistence/recurrence

Results

Ninety-three women treated for HSIL/CIN2-3 from July 2016 to July 2017 were included. Vaccination was recommended to all women at the moment of HSIL/CIN2+ diagnosis. All patients were treated using Loop Electro-Excision Procedure (LEEP). First visit after LEEP was performed at three months. From then, patients were followed-up every 6 months up to 24 months with cytology (Thinprep), HPV testing (Cobas), colposcopy and biopsy of necessary. The main outcome was histological SIL diagnosis confirmed during the follow-up visits (persistent/recurrent disease)

Conclusion

Forty-one of the women included (41/93; 44.1%) underwent HPV vaccination. First dose of the vaccine was provided between HSIL/CIN2+ diagnosis and four months after treatment either with 2-valent or 4-valent HPV vaccine. Margins were positive in 26.8% (11/51) of the cone specimens from the vaccinated women and 21.2% (11/52) of the cone specimens from the not-vaccinated women (p=0.346). No persistent/recurrent disease was diagnosed within the vaccinated women. Within the women who had not received the HPV vaccine, persistent/recurrent disease was diagnosed in 1.9% (1/52) of them (p=0.559). Mean time to persistent/recurrent disease diagnosis was 24 month

References

First generation HPV vaccines might reduce the risk of recurrent/persistent disease in women treated for HSIL/CIN2+ lesions. Larger well-designed studies to answer the question
as to the value of HPV-profilactic vaccine in the reduction of the risk of persistent/recurrent are warranted

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00056

IMPACT OF HPV VACCINATION WITH GARDASIL® IN SWITZERLAND

05. HPV prophylactic vaccines

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Background / Objectives

Gardasil®, a quadrivalent vaccine targeting low-risk (6, 11) and high-risk (16, 18) human papillomaviruses (HPV), has been offered to 11-14 year-old schoolgirls in Switzerland since 2008. The aim of our study was to evaluate its success and potential impact on cervical cancer screening, examining HPV genotypes in 18-year-old girls five years later (sub-study 1) and in outpatients participating to cervical cancer screening before and after vaccine implementation (sub-study 2).

Results

For sub-study 1, 3726 females aged 18 in 2013 were invited to fill a questionnaire on personal demographics and HPV risk factors and to provide a self-collected cervicovaginal sample for HPV genotyping and Chlamydia Trachomatis PCR. Personal data were evaluated by univariable and multivariable statistics. In sub-study 2, the proportion of the vaccine-type HPV among anogenital HPV was examined with archived genotyping data of more than 8050 outpatients participating to cervical cancer screening from 1999 until 2018. The yearly evolution of this proportion was evaluated by segmented logistic regression.

Conclusion

690 (18.5%) women participated to sub-study 1 and 327 (8.8%) provided a self-collected sample. Prevalence of Chlamydia trachomatis (4.6%) and demographics confirmed that the subjects were representative of sexually-active Swiss young women. Vaccine (five-year coverage: 77.5%) was preferentially accepted by contraceptive-pill users (p=0.001) and samples were mainly provided by sexually-active subjects (p<0.001). The proportion (4%) of the vaccine-type HPV in this population was lower than in sub-study 2 outpatients until 2015 (n=849, <26 years old) in the pre-vaccine era (25.7%). The proportion of the high-risk vaccine-type HPV decreased significantly (59%, p=0.0048) in the outpatients during the post-vaccine

era, yet this decrease was restricted to those aged less than 26 years (n=673, p=<0.0001) until 2015 (Jacot-Guillarmod M et al. BMC Infectious Diseases (2017) 17; 790). This was confirmed with the additional dataset encompassing 2016-2018.

References

The low proportion of vaccine-type HPV in 18-year-old females and its rapid decrease in young women participating to cervical cancer screening support the success of HPV vaccination to Switzerland. Our data suggest that cervical cancer screening is now entering a stage of reduced proportion of HPV16 and/or 18 in samples reported positive by cytology, leading to new screening strategies based on primary HPV testing.

00060

LONG-TERM ANTIBODY RESPONSE TO HUMAN PAPILLOMAVIRUS VACCINES: UP TO 12 YEARS FOLLOW-UP IN THE FINNISH MATERNITY COHORT

05. HPV prophylactic vaccines

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Background / Objectives

Most cervical cancers are caused by vaccine-preventable infections with human papillomaviruses (HPV). HPV prophylactic vaccines Gardasil™ and Cervarix™ both contain the major oncogenic HPV types 16 and 18, have been widely used for >10 years and are reported to induce high antibody levels and long-lasting protection. A head-to-head comparison of the antibody responses induced by the two vaccines has been performed only up to 5 years.

Results

About 3,500 Finnish females, who participated in phase III licensure trials of the Gardasil™ and Cervarix™ vaccines, consented to follow-up. Linkage with the Finnish Maternity Cohort found that they had donated >2,500 serum samples up to 12 years later. The most recently donated serum samples of 337 Gardasil™ and 730 Cervarix™ vaccine recipients were retrieved and serum antibody levels were determined using Pseudovirion- Luminex for HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59/68 and 73. To determine the level of a natural infection related antibodies, sera from women from Slovenian cervical screening cohort were analysed. Antibody levels were reported in international units (IU) in case of HPV16 and HPV18 and in-house units for the rest of the HPV types. Avidity of the antibodies in seropositive subject was evaluated using ammonium thiocyanate and reported using avidity index.

Conclusion

Post-vaccination HPV16 and HPV18 antibody levels remained stable and above natural infection-related antibody level for up to 12 years for most vaccine recipients. The median antibody levels were higher among Cervarix™ vaccine recipients in all time-windows from 7 to 12 years post vaccination ($p < 0.0001$). Seropositivity rate was higher in Cervarix™ group for all HPV types except HPV6/11/73 ranging from 1.1 fold (HPV16) to 3.3 fold (HPV45) indicating also existence of vaccine-related cross-protective antibodies. Avidity for HPV16 antibodies was 44% in Cervarix™ group and 30.5% in Gardasil™ group. Avidity for HPV18 antibodies was 33% vs. 28%. Higher avidity index in Gardasil™ group for HPV6 (9.3% vs. 27.6%) and HPV11 (10.9% vs. 34.5%) indicated higher quality of the vaccine induced antibodies compared to naturally derived ones. For other studied HPV types avidity indexes did not differ between vaccines tremendously varying from 4.3% (HPV51) to 19.6% (HPV 68) in case of Gardasil™ and 4% (HPV51) to 16% (HPV73) in case of Cervarix™.

References

The long-term stability of vaccine-induced antibody levels is in accordance with the high long-term protection reported previously. The observed significant differences in the antibody levels induced by the two vaccines imply that continued follow-up to identify possible breakthrough cases and estimation of the minimal protective vaccine-induced levels of serum antibodies is a research priority.

00073

Bivalent HPV Vaccine Effectiveness in a Japanese Population

05. HPV prophylactic vaccines

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Background / Objectives

April 2013, both the bivalent and quadrivalent HPV vaccines were included in the Japanese National Immunization Program. However, only two months later, the Japanese Ministry of Health, Labour, and Welfare suspended proactive recommendations for the HPV vaccine after unconfirmed reports of adverse events. One immediate consequence of the suspension was that vaccination uptake plummeted from over 70% to less than 1% within 12 months. A second consequence was that nonavalent HPV vaccine has not been licensed. We investigated bivalent HPV vaccine effectiveness (VE) against vaccine targeted types HPV 16 and 18 as well as against HPV 31, 45 and 52 those pointed out cross protection type.

Results

This study is a cross-sectional study recruiting women born after April 1993 attending for cervical screening at Niigata prefecture. We asked HPV vaccination status and sexual history (obtained on age at sexual debut, and number of sexual partners) in the questionnaire. We also confirmed HPV vaccination status from municipal records. Residual Pap smear specimens were collected for high-risk HPV (hrHPV) screening and genotyping test. We used Hybrid Capture 2 for hrHPV screening test and MEBGENTM HPV kit for hrHPV genotyping test. Data were analyzed using univariate and multivariate logistic regression analyses. VE was calculated as %VE=100x(1-OR).

Conclusion

The study enrolled a total of 2197 participants and included 1814 in the analysis. Of those analyzed, 1355 women had received the bivalent vaccine confirmed by municipal records. And 459 women were unvaccinated status by serf-reports and municipal records. In the univariate model, VE was statistically significant for pooled HPV 16/18 infections at 89.8%, (95% CI: 63.9% to 97.2%, p=0.001). VE of the women who were sexually naive at HPV vaccine initiation for pooled HPV 16/18 and HPV 31/45/52 was statistically significant at 95.5% (95% CI: 64.6%-99.4, p=0.0001)

and 71.9% (95% CI: 44.4%-85.8%, p=0.0002). VE for HPV 16, 31 and 52 individually was 94.3% (95% CI: 54.8%-99.3%, p=0.0005), 100% (p=0.008) and 63.1% (95% CI: 24.0%-82.1%, p=0.007). VE against HPV 18 and 45 individually was 100%, but did not reach statistical significance, due to the low overall number of infections. Adjusted for number of sexual partners, VE was 91.9% in HPV 16/18 (95% CI: 33.8%-99.0%, p=0.02) and 53.5% in HPV 31/45/52 (95% CI: 2.5%-77.8%, p=0.04).

References

We have shown high VE of the bivalent vaccine against vaccine-targeted hrHPV types 16 and 18 and significant cross protection against pooled hrHPV types 31, 45 and 52, which are associated with an additional 10% of ICC in Japan. This means the bivalent vaccine may be able to prevent around 80% of ICC in Japan. We hope Japanese government will resume proactive recommendations for HPV vaccine immediately.

00087

REDUCTION IN HPV16/18 POSITIVE HIGH-GRADE CERVICAL LESIONS IN A POPULATION OFFERED CATCH-UP VACCINATION

05. HPV prophylactic vaccines

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Background / Objectives

Australia introduced an ongoing, government-funded, school-based quadrivalent [HPV6,11,16,18] HPV vaccination program in 2007 for young girls 12-13 years of age, with a catch-up program to 26 years of age for women to the end of 2009. Using laser capture microdissection (LCM) and sensitive human papillomavirus (HPV) DNA genotyping, this study aimed to determine what impact the vaccination program has had on the proportion of HPV16/18-positive cervical intraepithelial neoplasia grade 3 (CIN3) and adenocarcinoma in situ (AIS) in young women of vaccine-eligible age living in Victoria, Australia, compared with pre-vaccination rates.

Results

Consecutive, histologically-confirmed CIN3 or AIS positive biopsies were collected between May 2011 and December 2014, from vaccine eligible women (born after 30th June 1981). Biopsy specimens were obtained from the Royal Women's Hospital Dysplasia Clinic (Parkville, Victoria, Australia) and VCS Limited (Carlton, Victoria, Australia). HPV genotypes present in whole tissue sections (WTS) were determined by a sensitive reverse hybridisation assay; RHA kit HPV SPF10-LiPA25, version 1 (Labo Bio-medical Products). Where the WTS was positive for multiple genotypes, lesions were isolated from biopsy material using LCM and genotyped. Cervical cytology samples from a pre-vaccine cohort with CIN3/AIS had been previously collected and genotyped using HPV Linear Array HPV Genotyping Test (Roche Diagnostics). Mixed genotype detections in the pre-vaccine sample set were resolved to a single lesion-attributed genotype using hierarchical attribution.

Conclusion

Overall, 743 cases were included. In the 18-25 year old group, the proportion of HPV16/18 cervical high-grade lesions decreased significantly over time from 69.4% in 2001-2005 (pre-vaccine), to 62.2% in 2011-2012, to 47.2% in 2013-2014 (p-trend=0.004). There was no significant change in HPV16/18 in the 26-32 year old group (p-trend=0.147).

References

In vaccine-eligible women aged 18–25 years old at time of biopsy, the proportion of CIN3/AIS lesions attributable to HPV16/18 was significantly lower than in a pre-vaccine-era cohort, giving an early indication that the Australian HPV vaccination program is effectively reducing cervical disease due to HPV16/18 infection.

00115

COMPARABLE VACCINE EFFECTIVENESS AGAINST CERVICAL INTRAEPITHELIAL NEOPLASIA AFTER VACCINATION WITH TWO OR THREE DOSES OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE

05. HPV prophylactic vaccines

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Background / Objectives

Although originally approved for three-doses, HPV vaccines were later approved for a two-dose schedule for 9-14 year olds. Registration of the two-dose schedule was based on immunobridging studies. We aimed to estimate the vaccine effectiveness (VE) of 2- and 3-doses of quadrivalent HPV vaccine (HPVV) against high-grade squamous intraepithelial lesion (HSIL) and cervical intraepithelial neoplasia grade 2 or higher (CIN2+) in screened young women.

Results

Data-linkage was performed between the population-based Cervical Cancer Screening Program (CCSP) and an immunization registry in British Columbia, Canada. Occurrence of HSIL and CIN2+ (CIN2 or CIN3) were compared in a screening cohort of women born between 1994-2005 who were unvaccinated or vaccinated with a recommended 2- or 3-dose schedule between 9-14 years of age

through a publicly funded school-based program. Incidence rates (IR, (95%CI)) and relative rates (RR) were calculated using adjusted Poisson regression. The VE was calculated as 1-RR *100%.

Conclusion

In total 26,059 women were included in our analyses; 12,762 were unvaccinated, 690 received a recommended 2-dose schedule and 12,607 a recommended 3-dose schedule. We observed a significant adjusted vaccine effectiveness (VE) against HSIL among women vaccinated between 9-14 years of age with either a recommended 2 (72.3%, 95CI 24.0-98.4%) or 3-dose schedule (37.7%, 95%CI 20.8-51.1%) compared to unvaccinated women. The IRs for CIN2+ among women vaccinated with two or three doses were 0.26 per 1000 person-years (PY) (95%CI 0.06-0.97) and 0.49 per 1000 PY (95%CI 0.36-0.66) respectively. There was no statistically significant difference in the relative rate of 2- compared with 3-doses for CIN2+ (adjusted RR 0.71 95%CI 0.07-6.66).

References

In this observational study, we did not observe a difference in VE after two-or three-doses of HPVV against both HSIL and CIN2+.

00355

PROTECTIVE EFFICACY OF THE AS04-HUMAN PAPILLOMAVIRUS (HPV)-16/18 VACCINE AGAINST NON-VACCINE HPV TYPES AMONG YOUNG WOMEN WITH CURRENT HPV EXPOSURE: POST-HOC ANALYSIS FROM A RANDOMIZED CONTROLLED TRIAL

05. HPV prophylactic vaccines

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Background / Objectives

HPV vaccines have been proven efficacious against vaccine-type infection among HPV DNA-negative women. It remains unclear whether L1 virus-like-particle-based prophylactic HPV vaccines are efficacious in protecting against high-risk (HR)-HPV types in women HPV DNA-positive for other HPV types at first vaccination.

Results

Women aged 18–25 years from Jiangsu province were randomized (1:1) to receive the AS04-HPV-16/18 vaccine (n=3,026) or $Al(OH)_3$ control (n=3,025) at months 0, 1 and 6 in a phase II/III, double-blind, randomized trial (NCT00779766).[1,2] In this post-hoc analysis, we evaluated the vaccine efficacy (VE) in specific subsets of women DNA positive to certain HR-HPV type(s) (i.e., 16/18/31/33/35/39/45/51/52/56/58/59/66/68) in the total vaccinated cohort. Subjects were included in this analysis if they were DNA negative for the HPV type(s) considered for efficacy and positive for any of other HR-HPV type DNA at baseline.

No initial serostatus was considered, except for analysis on women DNA negative and seropositive for HPV-16/18.

Conclusion

At baseline, DNA positivity was 15.3% for HR-HPV and 12.6% for HR-HPV excluding HPV-16/18. VE against 6-month persistent infection (6MPI) with HPV-16, HPV-18 and HPV-16/18 (any of those) among women DNA negative for HPV-16 and/or HPV-18 at baseline and positive for any other HR-HPV at baseline were 100% (95% confidence interval: 56.6–100%), 100% (18.7–100%) and 100% (75.4–100%), respectively. Similarly high VE (100% [33.1–100%]) against 12-month PI with HPV-16/18 was also observed in this population. In women who cleared prior infection to HPV-16/18 (DNA negative and seropositive to HPV-16/18), VE was 95.5% (72.0–99.9%) against HPV-16/18 6MPI.

We also noted substantial VE at 56.6% (16.2–78.6%) against incident infection associated with non-vaccine types HPV-31/33/45 among women positive for any of the highly prevalent HPV-39/51/52/58/66 and negative for HPV-31/33/35 at baseline. For women DNA positive for HPV-16/18 but negative for HPV-31/33/45 at baseline, efficacy against incident infection due to HPV-31/33/45 was 71.0% (27.3–89.8%).

References

Our findings lend support that for women with current exposure to any HR-HPV types at the time of first vaccination; vaccination with AS04-HPV-16/18 vaccine may protect against HPV infection caused by certain other high-risk oncogenic types. These results support AS04-HPV-16/18 vaccination of the general population without prescreening and vaccination among those with known HR-HPV infection status even for HPV- 16/18.

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00550

ONE DOSE OF HUMAN PAPILLOMAVIRUS VACCINE IS AS EFFECTIVE AS THREE FOR PREVENTION OF HIGH-GRADE CERVICAL LESIONS: NATIONAL COHORT STUDY

05. HPV prophylactic vaccines

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Background / Objectives

Prophylactic human papillomavirus (HPV) vaccines are highly effective at preventing pre-cancerous cervical lesions when given in a three-dose schedule. Some post-hoc trial data suggest that one dose prevents HPV infection. If one dose could prevent pre-cancerous cervical lesions, then global cervical cancer prevention would be greatly facilitated. We assessed the effectiveness of quadrivalent HPV vaccine by number of doses against cervical intraepithelial neoplasia (CIN) 2 or 3/adenocarcinoma-in-situ (AIS) in Australia up to seven years post vaccination.

Results

We created a linked dataset containing HPV vaccination history, cervical screening results, vital status and de-identified demographic details for all Australian women aged 15 or under when eligible for vaccine who had a screening test between April 2007 (when vaccination commenced) and 31 December 2014. We used Cox proportional hazard regression, adjusted a priori for age, socioeconomic status, and area of residence, to estimate hazard ratios of histologically confirmed CIN2/CIN3/AIS.

Conclusion

We included 250,648 women: 48,845 (19.5%) unvaccinated, 174,995 (69.8%) had received three doses, 18,190 (7.3%) two doses and 8,618 (3.4%) one dose. The

adjusted hazard ratio was significantly lower and not significantly different between dose groups compared to unvaccinated women (1 dose 0.63 (95%CI 0.51-0.79), 2 doses 0.60 (0.51-0.71) and 3 doses 0.60 (0.55-0.66).)

References

Despite differences in underlying characteristics of partially vaccinated women, we found that one dose was as effective as three at preventing high-grade disease. This finding supports decision makers to include one dose vaccination as a viable strategy when working towards the global elimination of cervical cancer.

FC 12. Vaccines 4

00131

THE HPV SEROLOGY STANDARDIZATION INITIATIVE: AIMS AND PROGRESS TO DATE AT THE FREDERICK NATIONAL LABORATORY FOR CANCER RESEARCH

05. HPV prophylactic vaccines

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Background / Objectives

Protection against Human Papillomavirus (HPV) infection after vaccination is believed to be mediated by HPV-specific antibodies. Antibody responses in HPV prophylactic vaccine trials have been assessed using different methods. The lack of standardized assays, procedures, and reagents accessible to the scientific community has precluded the comparison of different studies evaluating immunogenicity of HPV vaccines. With an expected increase in the number of trials relying on immunobridging for approval of new dosing schedules or vaccine formulations, there is a critical need for standardized measurement and reporting of immunogenicity to reliably assess non-inferiority of antibody responses and improve overall comparability between studies.

Results

The HPV Serology Laboratory at Frederick National Laboratory for Cancer Research was established in January 2017 to address this challenge, working with the National Cancer Institute (USA) and the Bill & Melinda Gates Foundation to lead standardization and harmonization efforts for HPV serological testing within HPV prophylactic vaccine trials.

The main goal is to expedite serology assay standardization by developing a critical set of qualified immunoassay reagents, including secondary standards and HPV Virus-Like Particles (VLP), as well as validated assays that will be made available to

the HPV scientific community. Furthermore, standard operating procedures for reagent production and qualification methods will be made accessible.

Conclusion

The HPV Serology Laboratory is currently developing qualified HPV VLP for the 9 HPV types included in currently licensed vaccines, HPV antibody secondary standards, serology-based proficiency panels, qualified serology assays, and testing guidelines. This work is being done in partnership with other HPV serology laboratories in the world.

References

Achievement of these aims will enable comparisons of data across different HPV vaccines and different studies and, therefore, it will facilitate vaccine development and implementation of new vaccine indications and new vaccine candidates.

00134

EFFECT OF BIVALENT HPV IMMUNISATION ON CYTOLOGICAL AND HISTOLOGICAL FINDINGS AT SECOND AND SUBSEQUENT SCREENS - A LONGITUDINAL STUDY

05. HPV prophylactic vaccines

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Background / Objectives

Scotland implemented school-based routine hr-HPV immunisation with Cervarix® at age 12/13 for women born in and after 1995, with catch-up immunisation for women born between 1990 and 1995. Women in the catch-up cohort have been screened since 2010, and routinely immunised women since 2015. As eight years have elapsed since the first immunised women began screening, it is possible to document the effect of HPV immunisation at the second and subsequent screening rounds for women in the catch-up cohort. This will be the first longitudinal data on the effect of bivalent HPV immunisation with direct linkage between immunisation and disease outcome

Results

Data on all cytology results and histology results, together with the number of doses of vaccine received, were extracted from the Scottish National Screening database for all women born between 1 January 1988 and 6 June 1996. Women born in 1988-1990 are largely un-immunised, women born in 1991-1994 are the catch-up cohort, and those born in 1995 and 1996 are the routinely immunised cohort. The reporting rates of cytological abnormalities and the histologically confirmed disease rates are compared between immunised and non-immunised women at their second and subsequent attendance for screening.

Conclusion

A total of 409,847 women were identified within the age range. A total of 127,855 women have had more than one cytology result recorded of whom 94,854 had negative cytology at their first screen. The cytology results and histological diagnoses taken from these women three years or more after their initial screen are shown in the table below, categorized by either no immunization or full immunization. Further detail on disease outcomes by birth cohort and time since first screen, stratified by immunisation status will be presented.

		Non immunised n (%)	Fully immunised n (%)
No. of women with negative initial screen		59792	30873
No. of cytology samples taken >=3 years after initial screen		104784	36486
Cytology	Negative	87033 (84.27)	31616 (88.05)
	Low grade	13726 (13.29)	3976 (11.07)
	High grade	2516 (2.44)	315 (0.88)
	? invasive	6 (0.006)	1 (0.003)
Histology	Negative	564 (0.54)	86 (0.24)
	HPV/CIN1	998 (1.30)	138 (0.38)
	HG CIN	2444 (2.34)	248 (0.68)
	Cancer	24 (0.023	2 (0.006)

References

In this analysis of women immunized as part of catchup and followed for up to 9 years, there has been a 64% reduction in the number of cytology samples reported with high grade cytology and a 71% reduction in the number of biopsies reported with high grade CIN. The number of cancers diagnosed is reduced by 75%. Bivalent HPV immunization is providing a substantial level of protection against cervical cancer and precancer that extends into the second round of screening and beyond.

00390

BIVALENT HPV VACCINE EFFECTIVENESS AGAINST ANAL HPV POSITIVITY AMONG FEMALE DUTCH STI CLINIC VISITORS

05. HPV prophylactic vaccines

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Background / Objectives

Anal cancer is responsible for the second largest HPV-related burden among women, with a steadily increasing share of the total disease burden due to rising incidence trends and absence of screening. HPV vaccines hold promise for anal cancer control, but data on vaccine effectiveness (VE) against anal HPV endpoints are scarce, especially among women. We estimated the VE of the bivalent HPV vaccine against type-specific anal HPV positivity among women visiting sexually transmitted infection (STI) clinics.

Results

We selected vaccine-eligible women from the PASSYON study, a biennial cross-sectional study among 16- to 24-year-old STI clinics visitors across the Netherlands. We aimed to include an anal swab of 30% of the women, independent of sexual risk behavior. Swabs were tested using the PCR-based assay SPF10-LiPA25. We compared the anal HPV positivity between self-reported vaccinated (≥1 dose) and unvaccinated women, and estimated the VE by a logistic mixed model against high-risk types HPV16/18/31/33/35/39/45/51/52/56/58/59 and low-risk types HPV6/11.

Conclusion

2246 women had been eligible for HPV vaccination of whom 548 (24.4%) provided an anal swab. Of the 548 women, 46.0% reported ever having had anal sex and 65.1% reported to be HPV vaccinated. Of the vaccinated women 0.8% tested

positive for anal HPV16/18 compared to 7.3% of the unvaccinated women, resulting in an adjusted pooled VE against anal HPV16/18 of 89.9% (63.0%–97.2%); 88.2% against HPV16 and 91.9% against HPV18. Moreover we calculated significant VE against HPV31 (72.9%) and HPV45 (100%).

References

We estimated high VE of the bivalent HPV vaccine against anal HPV16/18 positivity. Because these types are associated with almost 90% of all HPV-related anal cancer cases, vaccination provides a tremendous opportunity for anal cancer prevention.

00460

Impact of state legislation of human papillomavirus vaccination on vaccine uptake in the United States

05. HPV prophylactic vaccines

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Background / Objectives

In the United States, human papillomavirus (HPV) vaccination is universally recommended to adolescents at age 11. However, HPV vaccination rates lag behind those of other recommended adolescent vaccines. We identified states with laws regulating information dissemination or provision of HPV vaccination, and assessed the impact of these laws on rates of HPV vaccine uptake.

Results

The study period was October 2009-December 2014. We identified unvaccinated 11-year-olds adolescents from a commercial insurance claims database, and estimated rates of initiating HPV vaccination for each of 63 months during this period. We then searched the LexusNexus legal database for state laws around HPV vaccination that were passed during this period, and restricted analyses to adolescents in these states. We used segmented linear regression to estimate changes in levels of HPV vaccination (i.e. sudden change in rate), and trends of HPV vaccination (i.e. change in the slope of the rates) after passing the laws in each state. Model covariates included study month, time segment (pre- or post-legislation), time since passage of the legislation (months), and transformed sine and cosine functions of the rates to control for seasonality of vaccination.

Conclusion

Four states passed laws during the study period: Indiana (March 2013), Kentucky (February 2012), Missouri (July 2010), and Oregon (June 2013). Indiana's law allowed pharmacists to administer HPV vaccination. Laws in Kentucky, Missouri, and Oregon mandated education about HPV infection and cervical cancer. Only Oregon had a significant increase in HPV vaccination rates over the entire study period ($\beta=0.0319$, $p<0.0001$); however, rates slightly decreased after the law was passed in June 2013 through December 2014 ($\beta=-0.042$, $p<0.05$). Boys, but not girls, had significant increases in HPV vaccination rates in Indiana, Missouri, and Oregon over the entire study period. Boys in Missouri had significantly higher HPV vaccination rates after the law was passed in July 2010 through December 2014 ($\beta=0.11$, $p<0.05$). Urban adolescents accounted for the changes in HPV vaccination rates in Oregon, as no significant changes were observed in rural adolescents.

References

HPV vaccination rates in these four states did not show significant increases following the passage of pro-vaccination legislation. We saw positive trends among boys in Missouri, but across all states girls and rural adolescents did not have significantly higher vaccination rates after passage of legislation. School vaccination mandates without broad exemptions are needed to estimate their potential impact on HPV vaccination rates.

00465

HPV VACCINE PRESCRIPTION AND COMPLIANCE IN A COHORT OF WOMEN REFERRED FOR COLPOSCOPY

05. HPV prophylactic vaccines

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Background / Objectives

Human Papillomavirus (HPV) is a sine qua non factor for the development of cervical cancer. It is also associated with vulvar, vaginal, anal, oropharyngeal and penile cancer. Portugal introduced HPV vaccination of adolescent girls in 2008; since 2017 the schedule was changed to 2 doses of Gardasil 9 at age 10. Coverage rate of this program is of about 90%. Nevertheless, a significant part of older women are not vaccinated. The Portuguese Society of Gynaecology recommends vaccinating all women≤26 years old, and those with a diagnosis of a high grade cervical intraepithelial neoplasia (HSIL). The purpose of our work was to evaluate the prescription criteria and vaccination compliance in a cohort of women referred for colposcopy.

Results

Retrospective analysis of data from women who had their first appointment with one physician of our cervical pathology unit between January 2014 and July 2017 was performed. Demographic data (age, profession, civil status, immunosuppression, smoking), screening tests results, vaccine status, prescription and compliance were evaluated.

Conclusion

A total of 325 women were included, with ages ranging between 19 and 72 years old (median 40y, IC:32-47). Only 33 (10.2%) had already been immunized anti-HPV. Out of the others, the vaccine was prescribed in 34.9% of cases (n=102). Women with HSIL in histological evaluation were prescribed the vaccine in 51.5% (n=28) of the cases. The bivalent vaccine was prescribed in 79 cases (77.5%), followed by the nonavalent (n=18, 17.6%) and the quadrivalent (n=5, 4.9%). Half of the women (n=51) completed the 3 prescribed doses. Two women completed 2 doses; one only the first dose. In 23.3% (n=17) no dose at all was administered; the remaining 29 (28.4%) were lost to follow-up. Compliance was associated with the vaccine prescribed (bivalent 80.4% vs.tetravalent 50% vs.nonavalent 33.3%, p=0.003).A more severe Pap test

result showed a tendency towards higher compliance(>LSIL vs. ≤LSIL 83.3% Vs 64.6%[p=0.099]). On the other hand, no differences were found according to age, working status, smoking, contraception or indication for excision of the transformation zone.

References

Colposcopy is a good opportunity to promote HPV vaccination. It was recommended to one third of women, but compliance was low (50%). Price seems to be a key factor to explain this low uptake. This can explain why the bivalent vaccine was the more often prescribed and also why compliance was higher in this group. However, since the nonavalent vaccine is available, it is recommended as first option. Lower prices and/or state funding could increase the uptake of HPV vaccines.

00625

14 YEARS OF FOLLOW UP ON THE LONG-TERM EFFECTIVENESS AND IMMUNOGENICITY OF THE QUADRIVALENT HPV VACCINE IN 4 NORDIC COUNTRIES

05. HPV prophylactic vaccines

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Background / Objectives

FUTURE II, the pivotal efficacy study of the qHPV vaccine in young women 16-23 years of age was extended to investigate the long term effectiveness, immunogenicity and safety of the vaccine. Here, we present the end of study results after 14 years of follow up.

Results

During the base study participants received 3 doses of qHPV vaccine or placebo and were followed for ~4 years. Participants in the base study residing in Denmark, Iceland, Norway and Sweden were followed an additional 10 years through national health registries for effectiveness. Following the registry-based identification of all cases of high-grade cervical dysplasia, paraffin-embedded tissue blocks were retrieved and cut for thin-section HPV PCR testing. Slides were also created for pathology diagnosis adjudication. Vaccine effectiveness was estimated by comparing the observed incidence of HPV 16/18-related CIN2 or worse with the historical background incidence rate in an unvaccinated population (estimated by combining historical data from the national registries with survey data). An adapted Poisson Shewhart-based control chart approach for breakthrough disease incidence was used to monitor any waning of vaccine effectiveness. Per-protocol efficacy (PPE) analyses included participants who received 3 doses of qHPV vaccine in the base study and were seronegative and DNA negative for the relevant HPV type(s) prior to vaccination. Serum was collected at Years 5 and 10 of the LTFU study for

immunogenicity assessment. Geometric Mean Titers (GMTs) and % seropositivity to HPV 6/11/16/18 were assessed using cLIA and IgG LIA.

Conclusion

No new cases of the primary endpoint of HPV 16/18- related CIN 2 or worse were observed in the PPE population, which represented 2,121 subjects who contributed a total of 24,099.0 person-years of follow-up. A vaccine effectiveness of 100% was observed for at least 12 years post-vaccination with a trend of continued protection through 14 years post vaccination. There were no new cases of the secondary endpoint of HPV 6, 11, 16, 18-related CIN, vulvar cancer and vaginal cancer. Persistent anti-HPV 6, 11, 16, 18 GMTs were observed through 14 years of follow up, with seropositivity rates at end of study >90% for HPV 6, 11, and 18, and 52% for HPV 18 as assessed by cLIA, and >90% for all 4 HPV types, as assessed by IgG LIA.

References

The qHPV vaccine shows continued protection in women for at least 12 years with a trend of continued protection through 14 years of follow-up, and induces HPV 6, 11, 16, and 18 antibody responses that generally persist through 14 years post vaccination.

36. Public health

00330

PROJECTED IMPACT OF VACCINATION ON THE RISK OF HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION AND PRECANCEROUS LESION

36. Public health

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Background / Objectives

With the introduction of HPV vaccination, HPV-based screening programs will need optimization in the near future. Population-based data on the impact of vaccination on screening outcomes in the context of HPV-based screening are currently very limited. We predicted the impact of vaccination on five-yearly HPV-based screening on screen-detected HPV infections and CIN3+ under different vaccine scenarios.

Results

We included 21,287 women from a population-based screening trial with 14 years of follow-up (POBASCAM). We calculated cumulative incidences of screen-detected HPV infections and CIN3+, and positive predictive value (PPV) of a positive HPV test for CIN3+. The estimates for CIN3+ were based on a new statistical method linking type-specific HPV infections to type-specific CIN3+ [1]. We re-estimated the cumulative incidences and PPV after applying vaccine efficacy under three scenarios: i) bivalent vaccine, ii) bivalent vaccine with cross-protective efficacy, and iii) nonavalent vaccine. Analyses were performed separately for women aged 29-33 with a prevalent HPV infection at baseline (initial screening round) and women aged 29-58 with an incident HPV infection following a negative HPV test at baseline.

Conclusion

In total, 858 women had an HPV infection, leading to a cumulative incidence of 25.5%, which decreased to 18.9%, 15.2%, and 10.5% under the three different vaccine scenarios, respectively. The cumulative incidence of CIN3+ was 4.0% in absence of vaccination and decreased to 1.3%, 0.66%, and 0.18% under the three different vaccine scenarios, respectively. Amongst prevalent HPV infections, the PPV for CIN3+ decreased from 25.0% in absence of vaccination to 10.2% and 2.4% following bivalent and nonavalent vaccination respectively. Amongst incident HPV infections, the PPV decreased from 9.0% to 5.1% and 1.3%, respectively.

References

In the context of HPV-based screening, substantially lower cumulative incidences of screen-detected HPV infections and precancerous lesions must be expected in vaccinated women compared to unvaccinated. HPV vaccination further reduces screening efficiency reflected by the PPV, stressing the need for a screening program with differential risk of disease, for example by prolonging the screening intervals in vaccinated women.

References

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FC 13. New treatments

00522

EFFICACY OF A CARRAGEENAN-BASED LUBRICANT GEL IN INCREASING CLEARANCE OF HPV INFECTIONS IN WOMEN: INTERIM ANALYSIS OF A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

02. Epidemiology and natural history

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Background / Objectives

Carrageenan has been identified as a potent HPV infection inhibitor in preclinical studies. We aimed to evaluate the efficacy of a carrageenan-based lubricant gel in reducing incidence and prevalence of genital HPV infections among sexually active women.

Results

Between January 2013 and June 2017, 280 women aged 18 years and older were randomly assigned to a carrageenan (n=141) or a placebo (n=139) gel to be self-applied every other day for the first month and prior to and following each intercourse during follow-up. Assessments were done at baseline and at 0.5, 1, 3, 6, 9 and 12 months. 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 36 genital HPV types in self-collected vaginal samples. The primary outcome (reported previously) was the incidence of a newly detected infection by an HPV type that was not present at baseline. The second primary outcome was clearance of HPV types observed at baseline. We considered two definitions of clearance: 1 negative result and 2 consecutive negative results. We estimated hazard ratios (HR) and 95% confidence intervals (CI) using univariate Cox

models for the clearance of all HPV types. We used Cox models stratified by HPV types and clustered by participants for the clearance of individual HPV types to accommodate the correlated data structure.

Conclusion

67 (48%) of the 141 participants in the carrageenan and 80 (58%) of the 139 participants in the placebo arm were HPV positive at baseline. Baseline and follow-up characteristics were well balanced between arms for these participants. The median follow-up time was 9.2 months (interquartile range: 2.6-13.2). When considering clearance=1 negative result: 36 (54%) participants in the carrageenan and 34 (43%) participants in the placebo arm became HPV negative during follow-up (HR:1.45; 95% CI:0.90-2.32). When considering clearance=2 negative results: 23 (34%) participants in the carrageenan and 22 (28%) participants in the placebo arm became HPV negative (HR:1.23; 95% CI:0.69-2.21). When considering each HPV type individually, there were 174 infections at baseline in the carrageenan arm and 224, in the placebo arm. When considering clearance=1 negative result: 99 (57%) infections in the carrageenan and 123 (55%) infections in the placebo arm were cleared during follow-up (HR:1.35; 95% CI:0.92-2.00). When considering clearance=2 negative results: 62 (36%) infections in the carrageenan and 88 (39%) infections in the placebo arm were cleared (HR:1.10; 95% CI:0.74-1.63).

References

In our trial’s interim analysis, the use of a carrageenan-based lubricant gel was not associated with a significant increase in clearance of genital HPV infections.

00594

Adjuvant VACcination against HPV in surgical treatment of CINlesions,

06. HPV therapeutic vaccines

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Background / Objectives

ABSTRACT

INTRODUCTION: Human papilloma virus (HPV) causes cervical cancer. HPV vaccination is highly effective in primary prevention. There is less known about a possible effect of secondary prevention in women already infected with HPV. Our study proposes to investigate this efficacy in women with precursors of cervical cancer.

RESEARCH QUESTION:

Does HPV vaccination after Loop Electrosurgical Excision Procedure (LEEP), reduce the recurrence of Cervical Intra-epithelial Neoplasia II-III (CIN) lesions?

HYPOTHESIS

HPV vaccination after LEEP for CIN reduces recurrence.

STUDY DESIGN

Multicenter randomized double blind placebo controlled trial

STUDY POPULATION

Adult female patients with CIN II-III treated with LEEP and no prior vaccination for HPV.

INTERVENTION

HPV-vaccination or placebo after LEEP.

USUAL CARE/COMPARISON

Follow-up at 6 and 24 months after LEEP (HPV and cytology), according to the Dutch guideline.

OUTCOME MEASURES

Primary outcome: CIN II-III at 24 months

Secondary: high risk HPV presence, cytology results, number of re-interventions, cost-effectivity, adverse events and quality of life.

Tertiary outcome measure is efficacy of the vaccination after 5 and 10 yrs. Following completion of the trial, long term outcomes from the national screening program for cervical cancer will be obtained at 5 and 10 years.

SAMPLE SIZE

With a power of 0.8 and a two-sided alpha of 0.050, an estimated incidence of 8%(HPV-vaccine) and 3%(placebo)at 2-year follow-up for recurrence: 646 patients are needed. With 15% loss to follow-up, rounded up, a total of 750 patients has to be included.

DATA ANALYSIS

For the primary and secondary outcomes, the relative risk will be estimated comparing the vaccinated group to the placebo group, with 95% confidence intervals with chi-square or Fisher’s exact test for significance. All analyses will be intention-to-treat.

CURRENT STATUS

299/490

We recieved a grant at ZonMW GGG and expect the study to start on January 1st 2019

Results

This is a reseacrh proposal for a RCT. The trial will start around Janauary 1st 2019

References

A randomized trial for adjuvant vaccination with the nanovalent HPV vaccin in women with primary CIN is proposed.

07. Immunotherapy - Immuno-oncology - New treatments

00326

5% 5-FLUOROURACIL (5FU) TOPICAL THERAPY FOR THE TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) 2/3

07. Immunotherapy - Immuno-oncology - New treatments

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Background / Objectives

U.S. guidelines recommend 6 months observation for young women or surgical procedures for treatment of CIN 2/3.(1) There are no recommendations for medical management. Excisional procedures for cervical dysplasia have risks including adverse obstetrical outcomes. Some patients seek alternative therapies Prior studies of intravaginal 5FU as primary and adjuvant therapy to prevent recurrence of CIN 2/3 have been demonstrated to be effective.(2,3)

Results

This is a retrospective case-series from Jan 2014 to July 2018 of women offered options for management of CIN 2/3. These 25 women chose medical management with intravaginal 5FU (a 16-week course of biweekly self-applied intravaginal 5FU). Follow up included pap smear and colposcopy with biopsy at 6- and 12-months after initial diagnosis.

Conclusion

The majority of women were white (56%), privately insured (76%), and nulligravid (64%). The median age was 28 [22-44] years. All participants had a histologically confirmed CIN 2/3. Reasons for pursuing medical management are outlined in Table

1. In cases of women with severe immunosuppression that precluded surgical treatment, 5FU was prescribed while awaiting improved immune status. All women had follow-up at 6 or 12 months. By 6 months 5 women required surgical management for CIN 2/3 and at 12 months, 2 additional women underwent an excisional procedure. 72% (18/25) avoided surgical therapy within 12 months following CIN 2/3 diagnosis.

Table 1

Reason for 5FU	LEEP alternative, n (%)	14 (56%)
	Previous LEEP Procedure, n (%)	6 (25%)
	LEEP with positive margins, n (%)	3 (13%)
	Comorbidity precluding definitive treatment, n (%)	2 (8%)
Surgical Treatment in the 12 month period	Excisional Procedure, n (%)	6 (24%)
	Cryotherapy, n (%)	1 (4%)

References

Topical therapy with 5-FU may be an acceptable alternative for the treatment of cervical dysplasia in a motivated patient seeking an alternative to excisional procedures.

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00387

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

07. Immunotherapy - Immuno-oncology - New treatments

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Background / Objectives

Targeted treatment strategies against HPV-induced precancerous or cancerous lesions are still lacking. Analyses on the molecular biology of HPV-induced (pre-)cancer have revealed hypermethylation of both the host as well as the viral genome itself as a central oncogenic feature during HPV-related carcinogenesis. Specifically, hypermethylation of the HPV E2 binding sites (E2BS) in the upstream regulatory region of the HPV genome abrogates the regulatory function of the E2 protein, which allows uncontrolled overexpression of the HPV E6/E7 oncogenes. In addition, hypermethylation and associated silencing of tumor suppressor genes have been shown to occur in HPV-transformed cells.

Based on those observations we hypothesized that treatment of HPV-transformed lesions with demethylating agents could reverse this aberrant viral and host genome hypermethylation, down-regulate HPV oncogene expression and block uncontrolled proliferation, thereby representing a novel and targeted treatment approach.

Results

Seven HPV-transformed cell lines from the head and neck and the uterine cervix were treated with different concentrations of the demethylating agent 5-aza-2'-deoxycytidine. Dose- and time-dependent effects of the treatment on HPV oncogene

expression, cell proliferation and the induction of cell death and senescence were analyzed by a variety of assays. Three-dimensional (3D) tumor models (spheroids and co-cultures with normal keratinocytes) were generated from HPV-transformed cell lines and assessed for treatment effects. Transcriptome profile was analyzed in all treated cell lines using Illumina bead-chip technology.

Conclusion

A dose- and time-dependent down-regulation of HPV oncogene expression, significantly reduced proliferation and induction of apoptosis as well as cellular senescence was demonstrated in treated cell lines and 3D cultures. Transcriptome analysis revealed significant overexpression of cancer/testis antigens in treated cell lines, which has been associated with enhanced anti-tumoral immune response in previous in vivo studies.

References

Demethylating treatment represents a valuable treatment approach for HPV-induced (pre-)cancer by blocking cellular proliferation and potentially inducing an anti-tumoral immune response.

00521

MICROENVIRONMENT IN VAGINA AS A KEY-PLAYER ON CERVIX: VAGINAL MICROBIOTA COMPOSITION AND PREVALENCE OF HPV

17. Microbiome

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Background / Objectives

The vaginal microbiota has been associated to reproductive health and, more recently correlated with cervical carcinogenesis. The vaginal microbiota may modulate susceptibility to human papillomavirus (HPV) and other co-infections. Therefore, we evaluate the association between these infections and the vaginal microbiota.

Results

We evaluated the vaginal bacterial composition in 111 women from a private hospital, mean age 40.7±11.1 (range: 17-68 years old). Vaginal bacterial composition was characterized by deep sequencing of barcoded 16S rRNA gene fragments (V4) and HPV was identified using the Roche Linear Array® HPV genotyping test. The vaginal microbiota was categorized in community state type (CST). The cervical samples were obtained for cytology, HPV, *Ureaplasma parvum, Ureaplasma Urealyticum, Mycoplasma Genitaliumand 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.24). Significance was attributed if $P<0.05$.

Conclusion

The *Ureaplasma parvum* was the microorganism more prevalent (88.8%). For molecular diagnostic tests, the majority of women had a normal cytology (78.7%) and 30.9% presented HPV-positive, being all high risk-HPV types. Nevertheless, 60.6% had HPV-positive among women with abnormal cytology (P<0.001). High risk-HPV-positive women were younger (<33 years old) compared to HPV-negative (P=0.020). The younger women presented abnormal cytology (54.2%) and hrHPV-positive (44.1%) in relation to older women (P<0.05). The vaginal microbiota composition constituted by four CSTs: the majority presented CST I (n=63, 56.8%) and CST IV-B (n=26, 32.4%), followed by 2.7% in CST II and 1.8% in CST III. The CST-IV were more presented in older women (81.4%), although not statistically significant. Indeed, older women had a lower number of copies/mL of *Lactobacillus crispatus* in relation to younger women (P=0.001).

References

These preliminary results revealed that the clearance of virus in younger ages may be preponderant in the future development of cervical injuries. Indeed, the lower predominance of *L. crispatus* in older women may contribute to increased production of proinflammatory cytokines.

00423

THE iKNIFE AND ITS USE FOR THE TREATMENT OF CERVICAL ABNORMALITIES.

19. New technologies

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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), also known as the iKnife (intelligent Knife), 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.

Results

Cervical biopsies of 89 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.

Conclusion

The study showed correct classification with REIMS of almost 98%, with correct identification of cancer tissue of 83.3%, of CIN 100% and of healthy tissue 100%.

References

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 results of the iKnife. In addition to providing real-time information, thus reducing anaesthetic time, the iKnife 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 use in CIN to be ruled out into the colposcopy clinic.

00529

PHOTODYNAMIC THERAPY FOR HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA: A NEW POSSIBILITY?

19. New technologies

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Background / Objectives

5 to 22% high grade intraepithelial neoplasia (HCIN) can progress to invasive disease. The accepted HCIN treatment is excision of the transformation zone (ETZ). Photodynamic therapy (PDT)can induce cell death and stimulates local immune response, and may be a HCIN alternative treatment. The objective of this study was to evaluate Thin prep (TP) and PCR for high-risk HPV screening, in patients treated of HCIN (CIN 2) with PDT and ETZ, followed by 24 months.

Results

Controlled and randomized study with 40 women with histological diagnosis of HCIN (CIN 2), who collected TP and PCR, pre and post-treatment and followed up for 24 months. The patients were allocated into 2 groups with 20 women in each. ETZ was performed by loop excision procedure electrode. PDT was performed with the application of Methyl aminolevulinate cream 20% (MAL), 10 hours before the procedure and then, a single phototreatment with a LED tip. TP and PCR were collected pre and post treatment, every six months for 24 months.

Conclusion

The post ETZ follow up (24 months) showed that the TP was negative in 95% patients and PCR remained positive in 15% patients. The PDT group showed TP negative in 85% while 35 % patients remained PCR positive.

References

After 24 months of follow up PDT results resembled the ETZ and could be an alternative for HCIN, although dosimetry adjustment is still required.

00543

TOPICAL THERAPIES FOR TREATMENT OF HPV/CIN2-3

20. Diagnostic procedures / management

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Background / Objectives

High-risk human papillomavirus (hrHPV) infection is a precursor to cervical cancer, the leading cause of gynecologic cancer worldwide. Standard-of-care management for high-grade cervical dysplasia (also known as Cervical Intraepithelial Neoplasia or CIN 2-3) consists of surgical therapy which includes cryotherapy, laser therapy, and excisional procedures within the cervical transformation zone. Excisional options include Loop Electrosurgical Excision Procedures (LEEP) or cold-knife-cone (CKC).

Despite the overall success of excisional treatments and relatively low risk of immediate problems from these procedures, there are long-term side effects to consider, particularly in women of childbearing age. Women who undergo excisional procedures for cervical dysplasia potentially carry a 2 to 3-fold increased risk of preterm delivery compared with women without excision history. Women undergo psychological distress associated with the need for invasive procedures, and there are significant economic burdens associated with HR-HPV related disease. The development of a noninvasive patient-controlled mode of treatment has the potential to lower cost, long-term morbidity, and anxiety for women.

There are currently no medical therapies recommended to promote the clearance of HR-HPV infection or CIN. Cervical cancer is a cancer of economic, social, and educational disparities. A medical therapy option would overcome many of the barriers to the currently recommended surgical therapy – cost, requirement of skilled health provider for performing procedures, geographical barriers, patient fear of painful procedures – that are present in both developed and less-developed nations.This presentation will discuss literature on the efficacy topical therapy for management of HSIL.

FC 14. Methylation 1: From risk to triage

00435

THE PERFORMANCE OF FAM19A4/MIR124-2 METHYLATION ANALYSIS AS A TRIAGE TEST FOR HPV-SCREEN POSITIVE WOMEN AND AS A RULE OUT TEST FOR CERVICAL CANCER

12. Molecular markers

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Background / Objectives

Over the last years, the importance of primary hrHPV-based cervical screening has become clear, which has led to an adjustment of the cervical cancer screening program in various countries. Due to the low specificity of primary hrHPV screening, triage of hrHPV-positive women is essential to maintain a sustainable screening programme. DNA methylation analysis of cancer-related genes is a promising tool to identify hrHPV-positive women with cervical cancer or high-grade cervical intraepithelial neoplasia (CIN) in need of treatment. Host genes FAM19A4 and miR124-2 have been identified as attractive methylation markers and have a high potential for functioning as such a triage test. Furthermore, methylation analysis in small cervical cancer series has shown to detect all cervical carcinomas. A negative methylation test could thereby possibly be used as a rule out test for cervical cancer.

This project aimed to clinically validate the FAM19A4 /miR124-2 methylation analysis for the detection of high-grade CIN and cervical cancer in hrHPV-positive women participating in population-based cervical screening. Secondly, we aimed to evaluate the performance of the FAM19A4 /miR124-2 methylation analysis on a large series of cervical cancer.

Results

Archived HPV-positive cervical scrapes of hrHPV-positive women (age 29–61 years), who were enrolled in the VUSAscreen screening trial (ISRCTN64621295), were tested for FAM19A4/mir124-2 methylation analysis (QIAsure Methylation Test). The clinical performance in terms of sensitivity, specificity, NPV and PPV for CIN3+ was assessed. In addition, the positivity rate of FAM19A4/miR124-2 methylation analysis was determined in a large series of cervical cancer samples (scrapes or biopsies) from over 25 different countries worldwide.

Conclusion

In the screening cohort, the cervical scrapes of 276/979 (28.2%) hrHPV-positive women tested positive for FAM19A4/mir124-2 methylation. Cross-sectional performance of FAM19A4/miR124-2 methylation analysis among these women showed a CIN3+ sensitivity of 72.7% (95% CI: 64.8 - 80.6) at a specificity of 78.2% (95% CI: 75.4 – 80.9). An NPV of 95.2% (95C CI: 93.7 – 96.8) was found with a PPV of 32.3% (95% CI 26.8 – 37.8). In the cancer case series (n=513), a FAM19A4/miR124-2 methylation positivity rate of 98.6% was found.

References

FAM19A4/miR124-2 methylation analysis has a strong performance as a triage test for hrHPV-positive women in detecting CIN3+. Importantly, it detects virtually all cervical cancers and could therefore be used as a rule out test for cervical cancer.

References

Additional contribution.

16. Methylation

00104

DNA Methylation Panel for the Triage of HPV Positive Women in a Primary Screening Population.

16. Methylation

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Background / Objectives

Triage of HPV positive women is one of the key challenges facing HPV primary screening. Specific second round triage tests to avoid large numbers of unnecessary referrals to colposcopy are required. Host methylation factors have been repeatedly shown to be hypermethylated in cervical cancer/pre-cancer and have the potential to triage HPV positive women at high risk of cervical cancer. This study aims to investigate methylation of a specific panel of three markers [CADM1-M18, MALM1 and hsa-mir124-2] in HPV positive women. This study forms part of a larger CERVIVA HPV Primary Screening Study.

Results

In partnership with CervicalCheck, The National Cervical Screening Programme in Ireland, CERVIVA are undertaking a longitudinal HPV primary screening study evaluating triage strategies for managing HPV-positive primary screening tests. In total, 13,496 women attending for routine screening have been enrolled. HPV testing is performed using the Cobas HPV DNA test. HPV positive samples are tested for a panel of methylation specific biomarkers [CADM1-M18, MAL-M1, hsa-mir-124-2] via

Quantitative Methylation-Specific PCR and a Total Methylation Score (TMS) is calculated. Here we present a validation panel of 184 cervical cytology samples with confirmed histology for defining clinically relevant cut-off points that are being determined through ROC analysis for the detection of CIN3+. Testing of the HPV positive samples from the CERVIVA HPV primary screening study is underway.

Conclusion

The validation panel comprises of HPV positive and histology confirmed CIN1, CIN2, CIN3(n=50, 34, 50) and HPV negative/cytology no abnormality detected (NAD) (n=50). The data shows statistically significant differences in methylation scores for all markers (CAD-M1, MAL-M1, hsa-mir-124-2, and TMS(p=0.015, 0.016, <0.001, <0.001)) between those cases with CIN3 and NAD. Similarly, statistically significant differences were observed for all markers (CAD-M1, MAL-M1, hsa-mir-124-2, and TMS(p= 0.018, 0.018, 0.001, <0.001 respectively)) between cases of CIN3 and CIN1. ROC analysis shows an AUC of 0.910 when CIN 3 is compared to NAD. To date the methylation expression pattern of the three biomarkers has been assessed in 519 HPV positive primary screening cervical smears based on preliminary cut-offs, 36.6%(n=175/477) demonstrate elevated methylation scores. Elevated TMS was identified in 30% (65/218), 30.5% (63/206) and 60% (57/95) of cases with normal, LSIL/ASCUS and HSIL cytology respectively.

References

The Total Methylation Score generated by the combination of the methylation markers CADM1-M18, MALM1, and hsa-mir-124-2 shows promise in differentiating high grade lesions from normal and low grade lesions. Longitudinal follow up will be used to determine the clinical value of hyper-methylation in HPV positive women.

00108

EVALUATION OF A VALIDATED METHYLATION TRIAGE SIGNATURE FOR HUMAN PAPILLOMAVIRUS POSITIVE WOMEN IN THE HPV FOCAL CERVICAL CANCER SCREENING TRIAL

16. Methylation

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Background / Objectives

High-risk human papillomavirus (HPV)-based cervical cancer screening requires triage of HPV positive women to identify those at risk of cervical intraepithelial neoplasia grade 2 (CIN2) or worse, while avoiding over-treatment of women with transient HPV infections. HPV FOCAL is a randomized controlled trial which compared HPV (Intervention Arm) to liquid-based cytology (LBC) (Control Arm) screening for secondary prevention of cervical cancer. We evaluated whether methylation testing using the S5 classifier (based on HPV types 16, 18, 31, 33; and host gene EPB41L3) provides diagnostic triage performance similar to a more complex algorithm relying on cytology and HPV genotyping.

Results

Women aged 25-65 underwent screening. Based on known HPV/cytology results and pathology outcomes, groups of baseline specimens were randomly selected for S5 methylation testing (n=257). Group 1: 104 HPV positive (HPV+), abnormal LBC diagnosis (54 CIN2/3; 50 <CIN2); Group 2: 103 HPV+, normal LBC with HPV persistence at 12 mo. (53 CIN2/3; 50 <CIN2); Group 3: 50 HPV+, normal LBC with HPV clearance at 12 mo. (assumed <CIN2). Baseline specimens from eight women who developed invasive cervical cancers during or after the trial were also tested; these were not included in Groups 1-3. For Groups 1-3 combined, the S5 risk scores were calculated and the CIN2/3 relative sensitivity, specificity, and positive predictive value (PPV) were compared with other triage approaches. The methylation testing laboratory was blinded to HPV, LBC and histopathology results.

Conclusion

The S5 risk score showed a highly significant increasing trend with disease severity and HPV viral load. For CIN3, S5 relative sensitivity and specificity were: 93.2% (95%CI: 81.4-98.0) and 41.8% (35.2-48.8), compared to 86.4% (75.0-95.7) and 49.8% (43.1-56.6) respectively for combined abnormal LBC/HPV16/18 positivity (differences not significant); PPVs were 24.8% (18.3-31.5) and 26.2% (18.9-33.3) respectively. S5 was positive in baseline specimens from all eight women with cancers.

References

The S5 methylation risk score had high sensitivity and PPV for CIN3, surpassing US and European thresholds for colposcopy referral. S5 methylation signatures can identify most HPV positive women at increased risk of cervical cancer from their baseline screening specimens.

00150

METHYLATION BIOMARKERS FOR TRIAGE OF WOMEN BELOW THE AGE OF 30 WITH HPV POSITIVE SUREPATH COLLECTED SAMPLES.

16. Methylation

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Background / Objectives

Women below the age of 30 have a higher prevalence of oncogenic human papillomavirus (HPV) than older women, and implementation of primary HPV cervical cancer screening in this age group of women is challenged by the high HPV prevalence. Effective triage methods are therefore required to identify those with high risk of cervical high-grade intraepithelial neoplasia (CIN) and cancer, but equally importantly, to deselect HPV-positive women who are at low risk and can be safely referred for new testing at a later point in time. Here, we evaluate the QIAsure Methylation Test measuring the human biomarkers FAM19A4 and mir124-2, either or not in combination with oncogenic HPV genotyping, as a new potential triage method.

Results

Residual SurePath samples were collected from a population of 429 women positive for oncogenic HPV and cytology ≥ASCUS (age 15-29, average: 25 years). HPV testing was performed using the Onclarity HPV test (BD Diagnostics, Sparks, MD). Samples were reflex tested using the QIAsure Methylation Test (Qiagen, Hilden, Germany). All molecular testing was performed in concordance with manufacturer's specification. Women were referred to follow-up in concordance with Danish Guidelines. In total, 235 out of 429 included women had histology registered in the Danish Pathology Databank within 105-205 days (average 157 days).

Conclusion

A total of 163 women (38%) were hypermethylation positive using the QIAsure Methylation Test. Among the 235 women with histological follow-up, 72 had CIN1, 42 CIN2, 67 CIN3, and 54 had normal histology. When considering HPV and

methylation combined, the sensitivity was 70.1%, specificity 64.9%, PPV 44.3% and NPV 84.5% for ≥CIN3. Women with HPV 16,18,31,33&52 infections have been shown to have a higher risk of developing ≥CIN3 (data from Denmark), thus we considered the HPV genotype information with the methylation status as alternative triage strategy. The results showed a PPV and NPV of 54.2% and 62.1% respectively for the HPV16,18,31,33&52 group. For the NON(16,18,31,33,52) group the PPV and NPV were 8.7% and 97.7% respectively.

References

The resulting sensitivity, specificity, PPV and NPV of QIAsure Methylation Test indicates that this assay is an effective molecular reflex method for oncogenic HPV positive SurePath collected cervical samples from women below the age of 30, both alone and when combined with HPV genotype information. QIAsure Methylation Test can be considered as part of a unified molecular workflow for future molecular cervical cancer screening, saving laboratories the work load of reflex cytology on all oncogenic HPV positive screening samples.

00163

METHYLATION ANALYSIS OF HOST CELL GENES IN FIRST-VOID URINE TO DETECT CERVICAL PRECANCER LESIONS IN A REFERRAL POPULATION

16. Methylation

S. Van Keer [1], J. Pattyn [1], A.P. Van Splunter [2], A. De Smet [1], X. Van Ostade [3], W.A.A. Tjalma [4], M. Ieven [5], P. Van Damme [1], A. Vorsters [1], R.D.M. Steenbergen [2]

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Background / Objectives

Methylation of host cell and viral genes in urine has shown potential feasibility for cervical cancer triage and screening. As first-void urine (FVU) already ensures good high-risk (hr)HPV DNA agreement with paired cervical samples (CS), it offers the ability to test both primary hrHPV DNA and methylation markers in the same sample (one-step triage). Furthermore, due to its high preference, non-invasive character, and easy implementation, FVU-sampling is particularly interesting to reach non-participants in current screening programs. Hereto, in this study we report on hrHPV DNA prevalence and accuracy of host cell methylation markers in FVU.

Results

Paired FVU (Colli-Pee®, Novosanis) and CS (Cervex-Brush®, Rovers Medical Devices) were collected from 25- to 64-year-old women who were referred for colposcopy (NCT02714127) at the University Hospital Antwerp (UZA, Belgium). Cytology (ThinPrep® Pap Test, Hologic) and histology were investigated at UZA, followed by HPV DNA type-specific qPCR (AML, Belgium) on paired UCM (UAntwerp, Belgium)-buffered FVU and in PreservCyt® (Hologic) collected CS. Bisulphite converted DNA-extracts of UCM-buffered FVU were analysed for six methylation markers by quantitative methylation-specific PCR (Amsterdam UMC, The Netherlands). Statistics was performed using JMP Pro 13.

Conclusion

Ninety-five women (median 33 years; IQR: 29-43) were included, from whom 87 paired FVU and cervical HPV DNA results were available. A good hrHPV DNA agreement was observed between paired samples (Kappa: 0.62; 95% CI: 0.44-0.80), with a hrHPV DNA prevalence of 74 and 68% in FVU and CS, respectively. In FVU significant differences in host cell methylation levels were observed between high- and low-grade cervical abnormality based on cytology (4/6 genes), colposcopy (1/6 genes), and histological outcomes (2/6 genes) (Mann Witney U-test, $p<0.05$). Receiver operating curve (ROC)-analysis for the six methylation markers according to cytology (HSIL+), colposcopy (high-grade abnormality), and histology (CIN2+ and CIN3) showed a maximum area under the curve (AUC) of 0.73, 0.72, 0.72, and 0.86, respectively.

References

In FVU significant differences in host cell methylation levels were observed between high- and low-grade cervical abnormalities, as well as AUC's between 0.72-0.86 for at least one methylation marker (according to HSIL+/CIN2+/CIN3). Together with the good hrHPV DNA agreement between paired FVU and CS, these findings support the assertion that methylation analysis of host cell genes is feasible in FVU and holds promise as a molecular biomarker panel suitable for one-step triage. However, further study is ongoing and required to evaluate its clinical accuracy.

00170

HPV E4 EXPRESSION AND DNA HYPERMETHYLATION OF CADM1, MAL, AND MIR124-2 GENES IN CERVICAL CANCER AND PRECURSOR LESIONS

16. Methylation

M. Van Zummeren [1], W.W. Kremer [1], A. Leeman [2], M. Bleeker [1], D. Jenkins [2], M. Van De Sandt [2], J. Doorbar [3], D. Heideman [1], R. Steenbergen [1], P. Snijders [1], G.G. Kenter [4], W. Quint [2], J. Berkhof [5], C. Meijer [1]

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Background / Objectives

In this study we evaluate the expression of Human Papillomavirus (HPV) E4 protein (marker for the onset of a productive infection) and hypermethylation of host cell *CADM1*, *MAL*, and *miR124-2* genes (marker for an advanced, transforming infection) in cervical intraepithelial neoplasia (CIN) and cancer.

Results

One-hundred-fifteen cervical lesions were categorized by three pathologists into no dysplasia, CIN1, CIN2, CIN3 or cancer by classical histomorphological grading criteria, and by an immunoscore (cumulative value 0-6) grading system based on Ki-67 (score 0-3) and $p16^{ink4a}$ (score 0-3) expression. Lesions were immunostained for E4 protein and analyzed for hypermethylation of *CADM1*, *MAL*, or *miR124-2* genes. Expression of E4 and hypermethylation levels were related to CIN grade based on both classical and immunoscore grading.

Conclusion

Hypermethylation increased with severity of the lesion as defined by both classical histomorphological grading and immunoscore criteria, and was always present in

carcinomas (22/22). Extensive E4 expression decreased with increasing CIN grade and immunoscore, being most frequent in classically graded CIN1 or in lesions with cumulative immunoscore 1-3 and absent in carcinomas. High-grade lesions (CIN2/3 or immunoscore 4-6) showed less E4 expression, which was inversely related to an increasing hypermethylation. Extensive E4 expression, as observed in a small proportion of high-grade lesions (6/49 and 8/43 respectively), was mostly associated with a negative methylation marker status (5/6 and 7/8 respectively).

References

Our results illustrate the gradual transition of productive CIN (reflected by extensive E4 expression), to advanced transforming CIN (reflected by extensive hypermethylation) and cancer. Expression patterns of E4 and hypermethylation status of host cell genes, may be used to identify cervical lesions at risk for cervical cancer, providing a better guidance for clinicians on treatment decisions.

00215

DNA METHYLATION ANALYSIS IN URINE TO DETECT CERVICAL CANCER AND PRECANCER

16. Methylation

R. Van Den Helder [1], N.E. Van Trommel [1], A.P. Van Splunter [2], B.C. Snoek [2], D.A. Heideman [2], M.C. Bleeker [2], R.D. Steenbergen [2]

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Background / Objectives

Urine samples provide a potential alternative to physician-taken or self-collected cervical samples for cervical screening and is expected to increase the uptake of cervical screening programs. Various studies have shown the feasibility of hrHPV DNA testing on urine. However, screening by primary hrHPV DNA testing requires triage testing, for example DNA methylation analysis, to identify women in need of referral. We recently studied the feasibility of hrHPV DNA testing and host cell DNA methylation analysis in urine for detection of cervical cancer. Secondly, we initiated a clinical study (SOLUTION) to assess the performance of hrHPV DNA and DNA methylation analysis in urine samples for detection of CIN2/3 and cervical cancer using cervical samples as a reference.

Results

Urine samples and paired cervical scrapes were collected from 40 patients with cervical cancer patients and 44 female controls (feasibility study) and tested for hrHPV DNA presence and/or 6 previously identified methylation markers for cervical cancer. In the SOLUTION study, urine, cervical scrapes and/or self-collected cervico-vaginal specimens from 110 CIN2/3 patients and from 110 cervical cancer patients are collected and tested for hrHPV DNA and methylation markers.

Conclusion

Our feasibility study on cervical cancer patients showed a strong to near-perfect agreement between hrHPV DNA testing on urine and cervical scrapes (kappa=0.81). Also, DNA methylation levels in urine were moderately to strongly correlated to those

detected in cervical scrapes of the same patients (Spearman correlation coefficient 0.508 to 0.717). All 6 methylation markers were significantly increased in urine samples of cervical cancer patients compared to controls and revealed a good discriminatory power for cervical cancer in urine (AUC 0.74 to 0.89). SOLUTION study sample collection is currently ongoing and data on the performance of urine-based hrHPV DNA and DNA methylation analysis in comparison with cervical scrapes and cervico-vaginal samples will be presented.

References

Our studies indicate that urine-based hrHPV DNA and DNA methylation testing provides a promising strategy for the early detection of cervical cancer.

00280

DIFFERENTIATING CERVICAL PRE-CANCER FROM INVASIVE CANCER WITH THE S5 DNA METHYLATION CLASSIFIER

16. Methylation

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Background / Objectives

Background: Persistent infection with high-risk human papillomavirus (hr-HPV) is an important co-factor in cervical cancer development and is associated with DNA methylation on both human and viral genes. The S5 DNA methylation classifier, based on target CpG sites of the human gene EPB41L3, and viral late gene regions of HPV16, HPV18, HPV31 and HPV33 (Lorincz A et al., 2016) has demonstrated better performance for detection of CIN2/3 women than either HPV16/18 genotyping, cytology or combination. We tested the performance of S5 in detecting invasive cancers versus pre-cancers and quantified the degree of separation between normal/CIN1, CIN2/3 and invasive cancer S5 scores.

Results

Methods: Methylation status of the S5 selected CpG sites was tested in DNA extracted from formalin-fixed biopsies from the Scottish HPV Archive (UK, n=24) and PreservCyt collected exfoliated cervical cell samples from the Scottish HPV Archive (UK, n=48) and the International Agency for Research on Cancer (Spain, n=100). Samples were histologically defined as negative/CIN1 (n=33), CIN2/3 (n=65) and invasive cancer (n=74). DNA bisulfite conversion was carried out and followed by pyrosequencing for the 6 components of S5. Average methylation was calculated for each marker to define the S5 score.

Conclusion

Results: Methylation at all sites increased proportionally with disease severity with a Cuzick trend value of z = 9.2933 ($p < 2.2x10-16$). The separation of normal/CIN1 from CIN2/3 and from cancer was highly significant (Mann Whitney test, both $p < 0.0001$). S5 also showed highly significant difference between CIN2/3 and invasive

cancer from both IARC-Spain ($p < 0.0001$) and Scottish ($p < 0.003$) cohorts. Receiver operating characteristic (ROC) curves were used to assess the diagnostic potential of S5 in differentiating cancers from CIN2/3. The area under the ROC curve (AUC) was 0.86 (CI 95%: 0.7965 to 0.9131, $p < 0.0001$) with a sensitivity of 79.8% and a specificity of 83.1%, based on a cut-off at highest Youden J index.

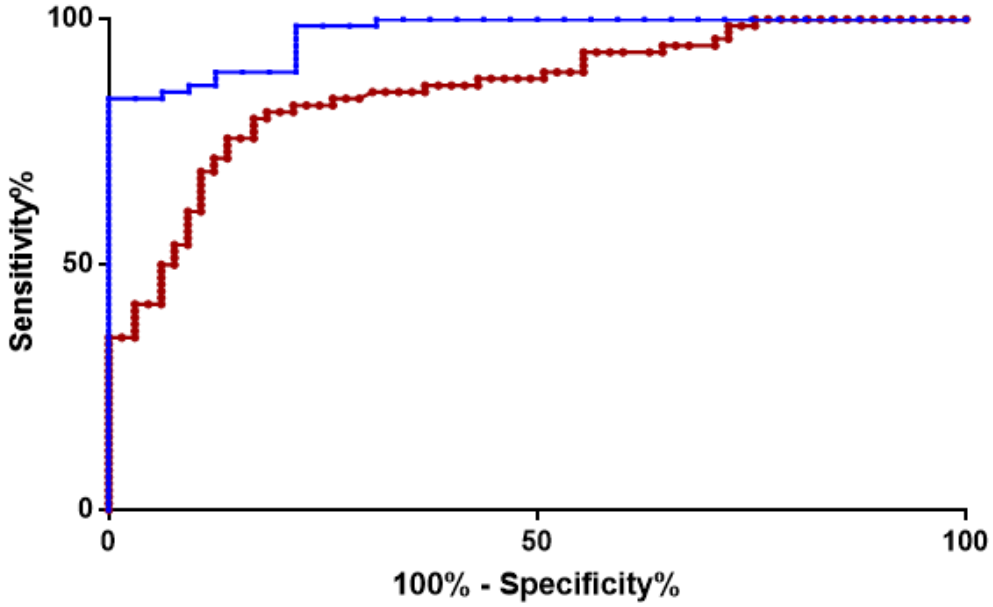
References

Conclusion: The S5 methylation classifier may be useful in cervical screening programs for differentiating pre-cancers from invasive cervical cancers in women infected with hr-HPV. Although the separation was very good, there is room for improvement in S5 by addition of new markers derived from an ongoing multi-omics study using next-Generation Sequencing.

References

Lorincz, A. T. et al. Validation of a DNA methylation HPV triage classifier in a screening sample. Int. J. cancer 138, 2745–51 (2016).

S5 methylation classifier performance ROC curves:
Normal/CIN1 vs Invasive Cancer (blue) and CIN2/3 vs Invasive Cancer (red)



Normal/CIN1 vs. Invasive Cancer
AUC = 0.9696
CI 95% (0.9433 to 0.9959)
p <= 0.0001
Sensitivity = 83.78%
Specificity = 100%
J derived-cutoff = 10.26

CIN2/3 vs Invasive Cancer
AUC = 0.8579
CI 95% (0.7965 to 0.9193)
p <= 0.0001
Sensitivity = 79.73%
Specificity = 83.08%
J derived-cutoff = 13.68

00334

FAM19A4/MIR124-2 METHYLATION ANALYSIS IN THE POBASCAM TRIAL WITH LONG-TERM FOLLOW-UP

16. Methylation

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Background / Objectives

DNA methylation analysis of HPV-positive cervical scrapes using *FAM19A4* and *miR124-2* genes has shown a good clinical performance in detecting cervical cancer and advanced CIN lesions in need of treatment. This study was conducted to assess the performance of *FAM19A4/miR124-2* methylation analysis in an HPV-positive screening cohort with long-term follow-up.

Results

Archived HPV-positive cervical scrapes of 1,040 women (age 29–61 years), who were enrolled in the POBASCAM screening trial (ISRCTN20781131) were tested for *FAM19A4/miR124-2* methylation. The nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA), was consulted to complete cytology and histology follow-up results over 14 years, comprising three screens (baseline, and after 5 and 10 years) .

Conclusion

The baseline scrape of 36.1% (n = 375) women tested positive for *FAM19A4/miR124-2* methylation and 30.6% (n = 318) had abnormal cytology (threshold borderline dyskaryosis or ASCUS). Within screening round capability of *FAM19A4/miR124-2* methylation to detect cervical cancer was 100% (11/11, 95% CI: 71.5–100). Kaplan–Meier estimate of 14-year cumulative cervical cancer incidence was 1.7% (95% CI: 0.66–3.0) among baseline methylation-negative and 2.4% (95% CI: 1.4–3.6) among baseline cytology-negative women (risk difference: 0.71% [95% CI: 0.16–1.4]). Results on the performance of *FAM19A4/miR-124-2* methylation analysis for CIN3+/2+ is currently ongoing and these data will be presented.

References

A negative *FAM19A4/miR124-2* methylation test provides a low cervical cancer risk in HPV-positive women of 30 years and older. *FAM19A4/miR124-2* methylation testing merits consideration as an objective triage test in HPV-based cervical screening programs.

References

Strooper LMA, Berkhof J, Steenbergen RDM, et al. (2018) 'Cervical cancer risk in HPV-positive women after a negative FAM19A4/mir124-2 methylation test: A post hoc analysis in the POBASCAM trial with 14 year follow-up', *Int J Cancer,* [Epub ahead of print]

00377

METHYLATION CAN PREDICT PROGRESSION OF CIN2

16. Methylation

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Background / Objectives

A substantial number of cervical cancer precursors, cervical intraepithelial neoplasias (CIN), regress without intervention. To date there is no method to predict their outcome, leaving treatment dependent on repeated examinations. A prognostic test could change the outline of cervical cancer screening and treatment of CIN. We investigated the ability of a DNA-methylation panel (the S5-classifier, composed of tumour suppressor gene EPB41L3 and HPV-targets) to discriminate between progression and regression among women with untreated CIN grade 2 (CIN2).

Results

Pyrosequencing methylation assays were run on exfoliated cervical cells from 149 women in a cohort study of active surveillance of CIN2 for 2 years in 18-30-year-old women in Helsinki University Hospital, Finland (ISRCTN91953024).

Conclusion

Twenty-five lesions progressed to ≥CIN3, 88 regressed to <CIN1, and 36 lesions persisted (CIN1/2). When cytology, HPV16/18-genotyping, and S5 at first visit were compared to clinical outcomes, S5 was the only marker associated with progression, odds ratio of 3.39 (95% confidence interval (CI) 1.35-8.50). S5 also showed significantly increased sensitivity compared to cytology in the outcome comparison of regression vs. persistence/progression. With both tests set at a specificity of 38.6% (95%CI 28.4-49.6) the sensitivities were 83.6% (95%CI 71.9-91.8) for S5 and 62.3% (95%CI 49.0-74.4) for cytology high grade squamous intraepithelial lesion (HSIL) (p=0.005). The highest area under the curve (AUC) was 0.735 (95%CI 0.621-0.849) achieved in the regression vs. progression outcome group with a combination of S5

and cytology ≥HSIL, whereas HPV16/18-genotyping did not provide additional prognostic information.

References

S5 classifier alone shows high potential as a prognostic biomarker to identify women with progressive cervical disease. S5 in combination with cytology ≥HSIL could be a useful triage test for women with CIN2 at risk of progression.

FC 15. Self-sampling 1

00064

FOR HIGH-RISK HPV TESTING THE SENSITIVITY AND SPECIFICITY OF A URINE SAMPLE EQUALS THAT OF A SELF-COLLECTED VAGINAL SAMPLE

10. Self-sampling

D. Oernskov [1], J. Augustenas [2], K.M. Jochumsen [3], I.M. Grunnet [3], A.W. Lykkebo [4], P.H. Steiner [4], D. Ejersbo [2], M. Waldstroem [2]

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Background / Objectives

Increasing focus has been added toward self-collected vaginal (SCV) samples as a means to increase the participation in the screening program for cervical cancer. Urine samples have also been tested for this purpose, but the knowledge on performance is still quite sparse.

The objective of this study is to:

in a colposcopic setting to examine the clinical performance of a self-collected sample (Evalyn Brush) from vagina (SCVS) and a urine sample compared to a sample taken by a physician for detecting high-grade pre-cancer lesions on cervix.

Results

Women referred to colposcopy at the gynecological departments at Lillebaelt Hospital and Odense University Hospital is being invited to participate in the study. Until now 270 women have been enrolled.

A urine sample and SCVS are performed by the women after a short instruction and before the medical examination. At the colposcopy an LBC sample (ThinPrep) and biopsies are taken. The urine, SCVS and LBC samples are analyzed for the presence of high-risk HPV using the Cobas HPV test, Roche. The biopsies are stained with H&E and p16 and are evaluated by gyneco-pathologists and used as the gold standard for the study.

Conclusion

The concordance between SCVS-urine and SCVS-LBC was high: 91% for both, while for urine-LBC it was 81%. For the sensitivity we found no significant differences: at CIN2+ it was 96%, 95% and 94% for SCVS, urine and LBC, respectively. At CIN3+ the values were very high: 100%, 100% and 97%, respectively.

Regarding specificity for SCVS, urine and LBC, at CIN2+ we found 42%, 45% and 44%, respectively and at CIN3+ 38%, 42% and 40%, respectively.

Among the women one carcinoma were identified and for this patient all three samples were positive for high-risk HPV positive.

References

These present data indicate that both the sensitivity and specificity of a urine sample equals that of a SCVS and the physician-taken LBC sample to identify CIN2+ cases. Updated data will be presented.

00213

PRIMARY HPV-BASED SCREENING WITH THE COBAS® HPV TEST ON SELF-COLLECTED CERVICOVAGINAL SAMPLES FROM UNDERSERVED GREEK WOMEN. PRELIMINARY RESULTS OF THE GRECOSELF STUDY.

10. Self-sampling

K. Chatzistamatiou [1], A. Tsertanidou [2], E. Mouchtaropoulou [3], K. Pasentzis [3], T. Moysiadis [3], A. Skenderi [4], S. Angelidou [5], E. Katsiki [5], V. Moschaki [6], A.M. Kaufmann [7], K. Stamatopoulos [3], T. Agorastos [2], S.G. Grecoself [2]

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Background / Objectives

To assess the performance of HPV-based cervical cancer screening in underserved Greek women using the cobas® HPV Test on self-collected cervicovaginal samples, compared with historical real-life results of cytology-based screening.

Results

The GRECOSELF project involved recruitment of women between 25-60 years old who do or do not attend cervical cancer screening and reside in rural areas of Greece. Sample size has been calculated at 12,700 women who would be enrolled over a period of 30 months starting May 2016. Women are contacted by midwifes, who comprise a nationwide network organized for the study purposes, at their place of residence, and are provided, after giving their written informed consent, with a self-sampling kit along with the necessary instructions. Each woman collects the specimen and fills in a questionnaire designed to give information about her cervical screening participation and outcome history, 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 as a pooled result. Women found positive for HPV are referred for colposcopy. Prior to colposcopy a physician-collected sample is taken to be tested for Cytology and Multiplex Genotyping (MPG). In case of abnormal colposcopic impression biopsies are taken. If biopsy is normal 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.

Conclusion

Between May 2016 and June 2018 12,758 samples were collected and 12,262 were tested (12,066 with valid results), of which 1008 (8.35%) were hrHPV positive and 168 (1.39%) were HPV 16/18 positive. To date, 702 colposcopies have been performed. Low grade disease (CIN1) was histologically detected in 67 cases, whereas high-grade disease (CIN2/CIN3/AIS) was diagnosed in 68 cases. Moreover, there had been two cases of Vaginal Intraepithelial Neoplasia (VaIN) and one case of invasive cervical adenocarcinoma. The prevalence of high-grade disease or cancer was 10.1% among the women referred to colposcopy. According to the historical data of the 71 women detected with CIN2/CIN3/AIS or cancer, 54 had a Pap test during the last three years (abnormal in only 5 cases), 12 before the last three years, and 5 did not have a Pap test in the past. The women with invasive adenocarcinoma reported a “normal” smear test during the last 3 years.

References

The preliminary report of the GRECOSELF study shows that HPV DNA testing with partial genotyping on self-collected cervicovaginal samples is a feasible and more effective than cytology cervical cancer prevention method for Greek women residing in rural areas.

00214

CERVICOVAGINAL SELF SAMPLING ACCEPTANCE AMONG UNDERSERVED GREEK WOMEN. A SURVEY CONDUCTED WITHIN THE FRAMEWORK OF THE GRECOSELF STUDY.

10. Self-sampling

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Background / Objectives

To assess the acceptance of cervicovaginal self-sampling using the Roche® self-collection device among Greek women residing in rural areas within the framework of the GRECOSELF project.

Results

Women recruited within the framework of the GRECOSELF project are between 25-60 years old, with different attitudes towards cervical cancer screening and reside in rural areas of Greece. Women are contacted by midwifes, comprising a nationwide network organized for the study purposes, at their place of residence, and are provided, after giving their written informed consent, with a self-sampling kit along with the necessary instructions. Each woman collects the specimen and fills in a questionnaire. Women positive for HPV are referred for colposcopy. The questionnaire is specifically designed to investigate cervical screening participation and outcome history, and self-sampling acceptance. The questions related to the latter were as follows: 1) “Did you understand the instructions given?”, 2) “Did you experience difficulties during self-sampling?”, 3) “Did you feel uncomfortable during self-sampling?” 4) “Did you feel pain during self-sampling?”, 5) “How sure are you that you followed the instructions correctly?” 6) “If self-sampling was an option where would you prefer to do it?”, 7) “Have you ever felt uncomfortable during physician-

sampling?” 8) “If physician and self-sampling were equally effective which one would you prefer?”, 9) “If physician and self-sampling were equally effective, would you check yourself more often?”

Conclusion

Between May 2016 and June 2018 12,758 women were recruited and 10,905 questionnaires were processed (85.5%). Most of the women (92.2%) stated that the self-sampling instructions were very clear or clear and 88.5% reported having very few or a few difficulties during self-sampling. Regarding discomfort and pain, most women reported having none of these (80.9% and 86.6% respectively). Moreover, 70,9% of the women felt very confident that they had followed the instructions correctly, and 61.8% reported that they would prefer to self-sample at home. A 34% of the women recruited stated that they had felt somehow or very uncomfortable during physician-sampling in the past. Concerning sampling preference, 64.7% of the women preferred self-sampling, 10.0% preferred physician-sampling and 19.4% had no preference. Finally, 68.9% of the women reported that they would be examined more often if self-sampling was equally effective to physician-sampling.

References

The survey conducted within the GRECOSELF study, regarding the acceptance of the self-sampling process in Greek women residing in rural areas showed that self-sampling is the preferred method, compared to physician-sampling, is easy to perform and causes minimal discomfort to women.

00323

EVALUATION OF BD ONCLARITY™ HPV ASSAY PERFORMED ON SELF-COLLECTED VAGINAL AND FIRST-VOID URINE SAMPLES AS COMPARED TO CLINICIAN-COLLECTED CERVICAL SAMPLES.

10. Self-sampling

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Background / Objectives

HPV testing conducted on self-samples has already been adopted by some countries to improve participation of hard-to-reach women to cervical-cancer screening programs. Indeed, the use of self-sampling may allow to overcome many social and cultural barriers that hinder women's participation to screening programs[1]. However, accuracy of testing self-samples as compared to clinician-collected samples needs to be evaluated using commercially available PCR-based clinically validated HPV detection kits[2]. The objective of this study was to evaluate BD Onclarity™ HPV Assay on testing vaginal and first-void urine samples as compared to clinician-collected cervical samples (gold standard).

Results

Clinician administered cervical and self-collected vaginal samples, using L-shape Endo/Esocervical and self-vaginal FLOQSwabs™ (Copan), and first-void urine samples, using Colli-Pee (Novosanis), are being collected from women referred to colposcopy for a recent history of cervical dysplasia, attending the Gynaecology Outpatients Clinic of San Gerardo Hospital (Monza, Italy). HPV detection is carried out using BD Onclarity™ HPV Assay on the fully-automated BD Viper™ LT System, able to detect 14 high-risk HPV (hrHPV) genotypes, according to manufacture's instructions. Sample cellularity is also evaluated using an "in house" quantitative real-time PCR detecting the human CCR5 gene.

Conclusion

Promising preliminary results have been obtained from the analysis of samples collected from the first 29 enrolled women. Concordant HPV detection for at least one hrHPV type has been demonstrated in 100% of vaginal self-collected and in 97% of urine samples as compared to clinician-collected samples. HPV16 resulted the most frequently hrHPV type detected followed by HPV31. Infections with multiple HPV types were shown in 38%, 27% and 24% of urine, cervical and vaginal samples, respectively. The majority of samples were found to have an adequate cellularity for all three specimens' types (mean values for urine, vaginal and cervical samples: 1.95E+06, 1.49E+06 and 3.43E+06 cells/sample, respectively).

References

HPV detection in self and clinician-collected samples showed a high degree of concordance using Copan's L-shape Endo/Esocervical and self-vaginal FLOQSwabs™ as well as Novosanis' Colli-Pee device with BD Onclarity™ HPV Assay. Although more studies are necessary to define the accuracy of HPV-testing on self-collected samples, these preliminary data demonstrate promising results for the use of HPV detection by PCR-based molecular methods on vaginal and first-void urine self-collected samples in cervical cancer screening programs.

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00327

OPTIMIZING A PROTOCOL FOR THE EVOLUTION OF VAGINAL SELF-COLLECTED SAMPLES USING COPAN FLOQSWAB© DEVICE FOR HPV DETECTION.

10. Self-sampling

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Background / Objectives

Vaginal self-collection samples for STI and HPV screening has been reported in publications since the year 2000, and its use has been advocated to improve women's participation to HPV screening programs. Devices, such as Copan FLOQSwab© (FS), Rovers Medical Evalyn®Brush and Eve Medical HerSwab, are available for self-collected samples. These are transported dry to the laboratory requiring elution in medium prior to testing. The elution procedure used for STI molecular testing usually follows manufacturers' specifications, while for HPV molecular assays different volumes such as 1, 2, 4, 4.6, 5,10 and 20 ml of SurePath or PreservCyt have been used for eluting self-collected samples. The objective of this study was to optimize the medium volume to elute FS vaginal self-samples, transported dry, for the detection of HPV with molecular assays as compared to professional cervical collection.

Results

Self-collected vaginal samples (SCVS) and physician administered cervical samples (PACS) were collected from 20 women referred to colposcopy at the Gynecology Clinic, San Gerardo Hospital. SCVS were collected first using FS, followed by PACS collection, using L-shape FLOQSwabs™ (Copan). The PACS were placed in 20mL PreservCyt (PC) (Hologic) while the SCVS were delivered dry to the Laboratory, of the University Milano-Bicocca. SCVS were suspended in 5mL PC Nucleic acid extraction was performed by NucliSENS®easyMAG (bioMérieux) and HPV was detected using AnyplexII™ HPV28 (Seegene). Sample cellularity was evaluated using an "in house" quantitative real-time PCR detecting human CCR5 gene. Later one ml aliquot of each SCVS was diluted 1:4 and retested for HPV.

Conclusion

Data obtained when performing HPV testing on dry-swabs eluted in 5 ml showed a very good concordance for high-risk HPV (hrHPV) detection between PACS and SCVS, with no evidence of inhibition. Excellent concordance in hrHPV detection was observed when comparing SCVS samples eluted in 5ml with those further diluted 1:4 (representing 20ml in PC). Adequate and comparable total sample cellularity for all PACS (mean value 2.67E+06 cells/sample) and SCVS (mean value 2.07E+06 cells/sample) samples was observed

References

Results showed a high degree of concordance in hrHPV detection between SCVS eluted in both 5ml and 20ml of PC as compared to PACS. This was further supported by the comparable and adequate total sample cellularity obtained from both PACS and SCVS using Copan FLOQSwab©. Standardization of an elution protocol for processing SCVS delivered dry to the laboratory would allow to reliably test samples for hrHPV detection and to compare results from different validation studies.

00339

NON-SPECULUM CLINICIAN SAMPLING FOR HPV TESTING TO INCREASE CERVICAL SCREENING UPTAKE IN WOMEN AGED 50 AND ABOVE

10. Self-sampling

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Background / Objectives

Women aged >65 account for a fifth of cervical cancers in the UK,(1) and around half of the deaths.(2) The majority are in women not adequately screened when aged 50-64,(3) but coverage continues to fall in this age group. Cervical screening with a speculum can become particularly uncomfortable after the menopause.(4) Self-sampling is a potential solution but 50%-70% of women worry about not taking a good sample.(5-8) HPV testing on clinician-collected vaginal samples without a speculum (non-speculum) is another possibility. Test performance would presumably be similar to self-sampling, but women would have the reassurance of a clinician-taken sample and could be particulalry attractive to postmenopausal women. Here we performed a cross-sectional study to examine test performance.

Results

Between Sep17-Jun18 non-speculum clinician samples and cytology (speculum samples) were collected from women aged 50-64 attending routine cervical screening in GP primary care and from women aged 50+ attending colposcopy (known or likely to be HPV positive). Samples were collected immediately before routine screening or colposcopy.

Sensitivity to high-grade disease (CIN2+) was assessed as the proportion HPV positive on non-speculum samples among histologically confirmed CIN2+ and among HPV positives on speculum samples. Specificity was assessed as the proportion HPV negative on non-speculum samples among women attending routine screening with <moderate dyskaryosis & <CIN2+.

Non-speculum samples were collected using a flocked swab (Copan 552C), transported dry, resuspended in ThinPrep prior to analysis using Roche Cobas4800.

Conclusion

Of 214 women attending routine screening, cytology samples could not be taken from 7 women because of pain/discomfort associated with the speculum. Of these, one had cytology subsequently collected, leaving 208 women with complete results: 198 were speculum HPV negative, all were <moderate dyskaryosis (none biopsied) and 96.5% (95%CI 92.9-98.6) were non-speculum HPV negative. Seven women were speculum negative/non-speculum positive – all cytology negative. The remaining 10 women were speculum positive/non-speculum positive (95%CI 69.2-100).

Of 46 women in colposcopy, 44 were speculum HPV positive, of these 90.5% (95%CI 72.6-94.8) were non-speculum positive. Of 7 with histologically confirmed CIN2+, all were non-speculum positive. Recruitment to this cohort will continue until Jan19.

References

Non-speculum clinician sampling for HPV testing is a viable option for older women. Preliminary data show that sensitivity is at least as good as speculum sampling for detecting HPV and CIN2+, but specificity may be lower. Findings should be confirmed in a larger study.

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00405

SELF-SAMPLING OF VAGINAL FLUID AND URINE FOR HIGH-RISK HUMAN PAPILLOMAVIRUS TESTING: AN OPTION FOR WOMEN PREVIOUSLY TREATED FOR HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA?

10. Self-sampling

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Background / Objectives

We assessed the performance of self-sampled vaginal fluid (VF) and first void urine (FVU) from women treated for CIN2+ for high risk HPV DNA (hrHPV) testing using a PCR-based clinically validated assay for cervical cancer screening by comparison to physician collected samples (REF).

Results

A prospective cohort of women with histopathology confirmed CIN2+ (N = 538); who underwent excisional treatment was followed-up at 6-months after treatment. Clinical assessment was carried out according to current standards of care including cytology, colposcopy and biopsy if the colposcopy indicated abnormal findings. VF (Qvintip, Aprovix) and FVU were collected by participants prior to sampling of a REF sample (PreservCyt, Hologic). Qvintip brush heads (air dried; stored at room temperature) were transferred into Cervi-Collect Tubes (Abbott) prior to testing. FVUs were mixed and transferred into Cervi-Collect Tubes within 30 minutes from collection and stored frozen until testing. Matched triplets of VF, FVU and REF samples were tested for the presence of hrHPV DNA (RealTime High Risk HPV, Abbott), following the manufacturer´s instructions.

Conclusion

A total of 484 matched triplets with valid PCR results were available for analysis. Good agreement of overall hrHPV (O) results was found between VF/FVU and REF samples (O-VF 88.6%, k 0.7; O-FVU 87.2%, k 0.6). High sensitivity for the detection of hrHPV in self-collected sample types versus REF was found with VF (90.5%; 95% CI:84.4–96.4), while the sensitivity for urine was (62.1%; 95% CI:52.4–71.9). In

contrast, the specificity of hrHPV detection in FVU was significantly higher versus REF (93.3%; 95% CI:90.8–95.8) than that in VF (88.2%; 95% CI:85.0–91.4). Sensitivity of hrHPV for the detection of residual/recurrent disease (HSIL/CIN2+) was 100% with REF samples and the specificity was 82.2% (95% CI:78.8-85.8). Somewhat lower sensitivity for the detection of residual/recurrent disease was found with VF (81.8%; 95% CI: 59.0 -100) and FVU (74.0%; 95% CI:46.4-99.1), respectively. Specificity of hrHPV for the detection of residual/recurrent disease (LSIL/CIN1) on FVU (83.7%; 95% CI:80.4-87.1) and was comparable to that of REF samples (83.7%; 95% CI:78.8 -85.8) and significantly lower on VF (74.0%; 95% CI:70.0 -78.0)

References

Good concordance of hrHPV detection between paired self-sampled VF/FVU and REF samples was observed in the ToC-setting. HrHPV testing of VF & FVU identified the majority of cases with residual/recurrent disease after 6 months detected by REF samples suggesting that hrHPV testing of both alternative sample types can be useful in follow up of women after treatment for high grade dysplasia. However, larger studies are required to confirm these findings

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00531

ACCEPTABILITY OF CERVICOVAGINAL SELF-SAMPLING IN CERVICAL CANCER SCREENING

10. Self-sampling

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Background / Objectives

Cancer of the uterine cervix is still a large public health problem mainly in poor regions,need for multiple consultation for follow-up,difficulty in quality control in the screening procedure and lack of resources to provide the necessary treatment. Some countries have explored alternative methods for the universalization and facilitation of access to cervical cancer screening, where self-sampling stands out. OBJECTIVE: To assess the results of vaginal self-sampling in cervical cancer screening and compare its acceptability against the collection performed by a health care professional.

Results

It is a prospective and cross-sectional study involving women over 21 years old. Participants (n = 214) were treated at the Gynecology outpatient clinic of the Hospital Universitário da Universidade de São Paulo (HU-USP) and Hospital das Clínicas das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP).

Conclusion

The participants had a mean age of 37.77 ± 11.06 years old, the majority of whom were white (62.3%) or brown (26.6%); 90.6% were in the menacme. Most women had only elementary school (literate, 45.7% and complete elementary school, 43.8%). In the obstetric history, 35.8% had 3 or more deliveries and 26.5% had only one delivery. The age of initial sexual activity was 16.78 ± 2.83 years (61.1% of cases from 15 to 18 years of age). Most women (35.6%) used a combined hormonal contraceptive method; condoms exclusively were used by 16.1% of the women. 71.8% were non-smokers and 71.9% reported not drinking alcohol; 98.5% reported not using any illicit drug. The following results refer to the questionnaire on the acceptability of cervicovaginal self-sampling. Regarding the understanding of the use

of the self-sampling brush (SSB), 50.48% considered it very easy; 45.71% considered it easy; 0.95% considered it a bit difficult; and none considered difficult. Regarding the effective use of the SSB, 38.10% considered it very easy; 55.24% considered easy; 3.81% considered it a bit difficult; and none considered difficult. During the use of the SSB, none reported much pain or discomfort; 4.76% reported pain or discomfort; 20.00% reported little pain or discomfort; and 72.38% reported no pain or discomfort. Besides that, none felt very embarrassed, 1.90% felt embarrassed, 19.05% felt little embarrassed and 76.19% did not feel embarrassed. Concerning the fear of getting hurt during the use of the SSB, 9.52% were afraid; 8.57% had little fear and 79.05% had no fear.

References

Most participants refer a good acceptability of cervicovaginal self-sampling with little or no discomfort, embarrassment, pain or fear during the procedure. Thus, due to its potential to increase the number of women screened, this procedure should be considered, above all, in public health policies.

FC 16. Self-sampling 2

00082

HPV SELF-TESTING/SELF-SAMPLING WILL SAVE INDIGENOUS LIVES

10. Self-sampling

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Background / Objectives

Māori (Indigenous) women experience unacceptably high rates of cervical cancer morbidity and mortality. Many Māori women find current cervical screening intrusive, resulting in low screening rates. ‘He Tapu Te Whare Tangata’ explored the acceptability of human papillomavirus (HPV) self-testing (/self-sampling) for never/under-screened Māori women to inform the New Zealand National Cervical Screening Programme.

Results

The study objectives were to explore under-screened Māori women’s reactions to HPV self-testing in hui (focus groups/interviews); survey Māori women about their HPV self-testing attitudes and potential behaviours; and canvasss key informants about HPV self-testing. A multi-disciplinary team, including elders and community based researchers (CBRs), conducted the Kaupapa Māori (by Māori, for Māori) research. CBRs ran hui with 106 eligible Māori women (aged >25 years, no screen in >4 years) in four regions, and arranged peer surveying (397 eligible surveys returned). The views of 16 key informants (KIs), including GPs and nurses, were canvassed.

Conclusion

A majority of survey participants were enrolled with a primary health care organisation (87%) and attended regularly (72%). However, they did not screen, with

‘whakamā’ (embarrassment/shyness/reticence) the most frequently given reason. Three in four participants said they were likely/very likely to do an HPV self-test, and 88% of women said they were likely/very likely to seek follow-up if required. Women and KIs agreed the delivery of test results should be tailored and that follow-up should be supported. Health practitioner cultural competence and empathy were emphasized.

References

When Māori women are engaged in the health system but do not screen, this is a system failure. The findings of this study indicate that with a culturally responsive, flexible HPV self-testing cervical cancer prevention programme (for example, available from community outreach workers at home visits and/or community centres, as well as opportunistically at clinics) many currently never/under-screened Māori women would be screened and followed-up if necessary. Recommendations to ensure optimum engagement with these women include: a strengths-based whole whānau approach to HPV education; and Primary Health Organisations working closely with community health providers to ensure standard recall, opportunistic in-clinic invitations to self-test, and targeted outreach. With well introduced HPV self-testing, many currently never/under-screened Māori women would be screened and followed up if necessary. HPV self-testing will save lives!

00249

EVALUATION OF SELF-SAMPLING FOR HPV AND STI TESTING AS AN ALTERNATIVE TOOL FOR WOMEN'S PARTICIPATION TO PREVENTION PROGRAMS

10. Self-sampling

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Background / Objectives

Self-sampling has been proposed as alternative tool to increase the participation of hard-to-reach women to prevention programs. The objective of this study was to evaluate the use of two different self-samples, vaginal and first-void urine samples, as compared to clinician-collected samples for the molecular detection of Human Papillomavirus (HPV) and other sexually transmitted infections (STIs) such as Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG), and Trichomonas vaginalis (TV).

Results

Physician administered cervical samples, self-collected vaginal samples using endo_eso_cervical and self-vaginal FLOQSwabs™ (Copan), and first-void urines using Colli-Pee (Novosanis) were collected from 82 women attending the Gynaecology Outpatients Clinic of San Gerardo Hospital (Monza, Italy) with a recent diagnosis of cervical dysplasia. Nucleic acids were extracted by NucliSENS easyMAG (bioMérieux) and HPV and STIs detection was carried out using AnyplexII™ HPV28 and AllplexTMCT/NG/MG/TV Assay (Seegene), respectively. Sample cellularity was evaluated by means quantitative real-time PCR detecting human CCR5 gene.

Conclusion

Preliminary data showed an adequate and comparable sample cellularity for all samples (mean value for urine, vaginal and cervical samples: 2.09E+06, 2.07E+06 and 2.67E+06 cells/sample, respectively). An optimal concordance for at least one high-risk HPV type detection compared to cervical sample (gold standard) was

demonstrated for both self-samples (k=0.95). HPV16 resulted the most frequently hrHPV type detected. HPV co-infections were shown in 51%, 55% and 64% of cervical, vaginal and urine samples, respectively. A higher STIs positivity was found in self-sampling samples compared to physician-collected samples with Chlamydia trachomatis being detected in 4% and 5% of clinician administered and self-sampling, respectively.

References

Cellularity of both self-collected and clinician-collected cervical samples using Copan endo_eso_cervical and self-vaginal showed comparable results and both HPV and STIs detection using molecular methods demonstrated a high degree of concordance. These data reveal promising results for the introduction of self-collected samples in cervical cancer and sexually transmitted infections screening programs.

00297

COMPARISON OF DIFFERENT SELF-SAMPLING DEVICES FOR SEXUALLY TRANSMITTED INFECTIONS (STI) AND HUMAN PAPILLOMAVIRUS (HPV) DETECTION USING MOLECULAR METHODS

10. Self-sampling

I. Sechi [1], M. Martinelli [2], R. Musumeci [2], G. Brenna [2], E. Calaresu [2], F. Perdoni [2], A. Piana [1], A. Cossu [1], N. Muresu [1], C. Cocuzza [2]

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Background / Objectives

Vaginal Self-collection for STI and HPV screening is generally well accepted by women, although this may raise patients' concerns on their ability to collect adequate samples, particularly as some devices may require more extensive manipulations. Copan developed the vaginal self-collection FLOQSwab©(FS) suitable for STI and HPV screening with molecular assays.

The objective of this study was to compare FS to two other self-collection devices: Evalyn®Brush (EB) (Rovers Medical) and HerSwab(HS) (Eve Medical) for:

1. Performance of devices for HPV and STI molecular detection as compared to professional cervical collection;
2. Ease of use of devices for sample collection;
3. Costs of collection devices.

Results

Self-collected vaginal samples (SCVS) and physician administered cervical samples (PACS) were collected from women referred to colposcopy at the Gynecology Clinic, San Gerardo Hospital. SCVS were collected from 20 patients each using FS, HS and EB alternating the order of collection for each device, lastly PACS were collected. Self-collection procedures, provided by each manufacturer, were used and a questionnaire to evaluate the ease of use, self-sampling level of satisfaction and acceptability was completed by participants.

The PACS were placed in 20mL PreservCyt (Hologic) while the SCVS were delivered dry to the Microbiology Laboratory, University Milano-Bicocca. All SCVS were suspended in 5mL PreservCyt. Nucleic acid extraction was performed by means NucliSENS®easyMAG (bioMérieux) and STI and HPV was detected using AnyplexII™ HPV28 and Allplex™ CT/NG/MG/TV Assay (Seegene). Costs analysis for purchasing each self-collection devices was performed.

Conclusion

Data obtained showed an excellent STI and high-risk HPV (hrHPV) detection concordance between PACS and SCVS using both FS and HS (k=0.95). A good concordance (k=0.75) was demonstrated comparing cervical samples and both FS and EB samples for both STI and HPV. Most women did not experience any problems when using the 3 different self-collection devices. Ninety-two percent of women declared to prefer self-sampling to undergoing gynaecological visit for testing. Cost analysis revealed that whilst FS is available at 0,60 - 0,70 Euro, EB and HS are approximately 4 and 9 times more expensive.

References

Preliminary results showed a high degree of concordance in STI and HPV detection between SCVS and PACS. Self-sampling, was preferred by women over PACS. In particular FLOQSwabs®, proved to be easier to use and cheaper as compared to other devices. Self-collection appears to be promising alternative to improve women's participation to STI and HPV screening programmes.

00369

VAGINAL SELF-COLLECTION VERSUS CERVICAL CLINICIAN-COLLECTED SAMPLES FOR CERVICAL CANCER SCREENING: WHAT WOULD YOU CHOOSE? RESULTS FROM SELF SAMPLING SATISFACTION QUESTIONNAIRES

10. Self-sampling

G. Fantacci [1], C. Sani [1], E. Burroni [1], A. Mongia [1], S. Bisanzi [1], G. Pompeo [1], F. Cellai [1], A. Iossa [2], G. Grazzini [2], C. Di Pierro [2], E. Cavazza [2], C.B. Visioli [3], C. Nicolai [4], L. Solfanelli [4], A. Lombardi [5], F. Carozzi [1]

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Background / Objectives

An ongoing ISPRO (Florence) research project on self-collection involved 5200 “non-responder” women: some performed sample collection at the clinic; others received at home “Dry” self-sampler device FLOQSwab®(Copan) or “Wet” device, containing 1 ml of MSwab®(Copan) both together with a satisfaction questionnaire, designed with the purpose to investigate the acceptability of self-collection rather than of clinician-collection of the sample.

Results

The questionnaire consists of three areas: easy of use of the self-collection system, possible physical problems (pain or bleeding) related to the use of the self-collection device and expression of preference between self-collected and physician-taken cervical sample. The results were evaluated with an average score, based on the scores given to each question. “Chi-square test” was used to compare the difference between scores.

Conclusion

The questionnaire was compiled by 99% of women who attended self-sampling: 90.6% found the self-collection system very easy/easy and 97.8% found easy the collection procedure and understood the instructions for the withdrawal. Related to the self-collection, discomfort or bleeding, 92.2% had no pain; finally, 81.4% of women preferred self-collection method rather than cervical clinician collection. There was no significant differences ($p>0.05$) between the two arms for type of self-sampling device methods or by level of education.

References

From questionnaire, it emerges that most of women found self-collection procedure easy or very easy; they did not report any particular pain, discomfort or bleeding and preferred self-collection, “Dry” or “Wet”, for reasons of time and comfort. Therefore, there is a good acceptability of the self-sampling system.

00381

URINARY HPV DNA TESTING AS A TOOL FOR CERVICAL CANCER SCREENING IN FRANCE: AN UPDATE OF THE CAPU-3 STUDY

10. Self-sampling

C. Lefeuvre [1], A. Pivert [1], T. Bickert [1], M. Boitel [1], R. Houlet [1], A. Marchand [1], G. Michel [1], P. Veillon [1], F. Lunel Fabiani [1], A.S. Le Duc Banaszuk [2], A. Ducancelle [1]

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Background / Objectives

In France, cervical cancer screening is currently based on cytological examination of a Pap smear for women aged 25 to 65, but screening coverage is unsatisfactory. Previous studies in our lab have shown that urinary HPV testing for high-risk human papillomavirus (HR HPV) testing increases rates of compliance (1,2). Since November 2016, the CapU-3 study aims to invite 13,000 women aged 35 to 65 who did not performed a Pap smear over the past 7 years in Maine et Loire department. 500-700 letters proposing an at-home urinary HPV testing are sent monthly. With the letter, the women receive an urinary HPV DNA testing information note, a letter of informed 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.

In collaboration with Cap Santé 49, we conducted a pilot study to offer urinary HPV testing for women who don't have regular cervical smear in order to increase the cervical cancer screening coverage in our department.

Results

HR HPV detection is performed using a real-time PCR technique (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.

Conclusion

After exclusion (past hysterectomy or refusal), the participation rate is 14.2% (CI95%: 13.6%-14.8%).

Out of the 1637 analyzed specimens, 1450 and 168 were negative and positive for at least 1 HR HPV respectively. HR HPV others than HPV 16 or HPV 18 were mostly detected as HPV 53 (15.8%; CI95%: 11.3%-21.7%) and HPV31 (10.5%; CI95%: 6.9%-15.7%).

Invalid results occurred in only 19 samples.

Among the cervical smears performed in positive patients, 4 high-grade cytological lesions have already been detected.

References

Because home HPV urinary testing is non-invasive and do not require medical attention, this method may be an alternative for women who are reluctant to use Pap smear. Furthermore, 70.5% of the HPV-positive women included in the CapU3 study benefited from a Pap smear collected by a clinician during follow-up. So, the urinary HPV test could be an alternative to the usual screening by cervical smear thus extending screening coverage in our department.

References

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00452

Temperature and time stability of self-collecting samples in Japan where the temperature sometime reaches over 35-40 degrees Celsius in summer

10. Self-sampling

M. Ito [1], H. Konishi [2], K. Katagiri [3], Y. Shimizu [4], Y. Ohashi [5], Y. Matsuyama [6]

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Background / Objectives

In the past few years, we have conducted some trial studies to improve the coverage of the population based cervical cancer screening using self-collecting HPV test in Japan because Japanese coverage of the screening is so low that incidence and mortality of cervical cancer is increasing especially in younger generations such as late 20s and early 30s. Acceptance of the participants was widely accepted and they preferred the self-collecting HPV test much more than the conventional cytology test the Japanese government recommends. We use Evalyn Brush as a self-collection device which the Netherlands and Denmark use for non-responding people for their national cervical cancer screening programs. However, in Japan, the summer temperature often soars to over 35 degrees Celsius and has reached 40 degrees in some parts this year. The temperature of public mailboxes in some towns has reached around 50 degrees Celsius. The samples of self-collecting HPV tests are sent to the testing laboratory through the postal service and could be exposed to sweltering environments.

We want to make sure if the self-collecting HPV test sample using Evalyn Brush is stable in a high temperature environment such as a summer in Japan. If we can find out how high environment temperature the sample can sustain, it must be useful information in other parts of Asia.

Results

We get 2 samples from the same participant via physicians using Evalyn Brush. After collecting and sending the samples to the testing laboratory, one sample is tested as a normal test and another one is kept in 4 conditions and tested as an experimentally

high temperature environmental test; First in 50 degrees Celsius for 2 weeks; Second in the same temperature for 4 weeks; Third in 30 degrees Celsius for 2 weeks; fourth, in the same temperature for 4 weeks. We are planning a collection sample size of approximately 20 samples per group for a total of 80 samples. We compare the results of the samples from the same participant and confirm if there is a difference.

Conclusion

Our study is ongoing as of August 2018.

References

We will be able to report the entire result at the EUROGIN 2018 conference.

00459

An effective 3-gene methylation classifier for direct triage on hrHPV-positive self-samples

10. Self-sampling

W. Verlaat [1], L. Verhoef [1], B.C. Snoek [1], D.A.M. Heideman [1], S.M. Wilting [1], P.J.F. Snijders [1], P.W. Novianti [1], A.P. Van Splunter [1], C.F.W. Peeters [2], N.E. Van Trommel [3], L.F.A. Massuger [4], R.L.M. Bekkers [4], W.J.G. Melchers [5], F.J. Van Kemenade [6], M.C.G. Bleeker [1], J. Berkhof [7], M.A. Van De Wiel [7], C.J.L. Meijer [1], R.D.M. Steenbergen [1]

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Background / Objectives

Offering self-sampling of (cervico-) vaginal material for high-risk HPV (hrHPV) testing is an effective method to increase the coverage in cervical screening programs. However, an additional triage test directly applicable to self-sampled material is necessary to identify hrHPV-positive women at risk for progression to cervical cancer. Since cytology cannot be reliably performed on self-sampled material, there is a need for molecular triage markers. Candidate molecular disease markers for triage testing involve the host cell epigenetic changes, such as DNA hypermethylation, that following a transforming hrHPV infection drive progression to cancer. Earlier work has shown that methylation analysis virtually detects (virtually) all cervical carcinomas and advanced CIN2/3 lesions. Longitudinal outcome data in an hrHPV-positive screening cohort illustrate a low 14-year cervical cancer risk among baseline methylation-negative women as compared to baseline cytology–negative women. Here, we set out to identify and validate a DNA methylation classifier for detection of cervical precancer (CIN3) and cancer, applicable to self-samples.

Results

We determined genome-wide DNA methylation profiles of 72 hrHPV positive self-samples, from screening non-attendees using the Infinium Methylation 450K Array, and further evaluated the selected DNA methylation markers by multiplex quantitative methylation-specific PCR (qMSP). Logistic regression analysis was performed to build a DNA methylation classifier for CIN3 detection applicable to self-samples. For validation, an independent series of HPV-positive self-samples was used.

Conclusion

Genome-wide DNA methylation profiling revealed 12 DNA methylation markers for CIN3 detection. Multiplex qMSP analysis of these markers in large series of self-samples yielded a 3-gene methylation classifier (ASCL1, ST6GALNAC5 and LHX8). This classifier showed a good clinical performance for CIN3 detection in HPV-positive self-samples in the validation set. Importantly, all self-samples from women with cervical cancer scored DNA methylation-positive.

References

A highly effective 3-gene methylation classifier for direct triage on hrHPV-positive self-samples was identified using a genome-wide DNA methylation profiling. Our findings indicate that a transition towards full molecular self-screening in hrHPV-based cervical screening programs is feasible. The study findings are currently evaluated in a self-sampling study cohort from the regular screening population.

00316

UTILITY OF URINE ONCOGENIC HPV TESTING FOR DIAGNOSIS OF CIN 2+

13. Screening methods

L. Rahangdale [1], A. Knittel [1], C. Edelman [2], B. Sanusi [3], S. Tulenko [4], L. Romicki [5], V. Sivaramam [5], S. O'connor [6], J. Nelson [7], J. Schmitt [8], B. Faherty [9], K. Chesko [9], L. Vaughan [9], J.S. Smith [10]

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Background / Objectives

Cervical cancer incidence and death are highest among medically underserved women in the U.S. Urine testing for hrHPV (high risk) as a primary screening test may be more acceptable and accessible to at-risk women. We aimed to assess the validity of testing for oncogenic HPV in urine for the detection of high-grade cervical precancer (CIN2+).

Results

Self-collected urine, self-collected cervico-vaginal and physician-collected cervical samples were obtained from women undergoing clinically-indicated cervical colposcopy or excisional procedures at the University of North Carolina and Duke University medical centers. Women with normal cytology and positive hrHPV test results were also recruited and underwent colposcopy. Samples were tested for high risk (HR) HPV E6/E7 DNA using the BD Onclarity HPV Assay (Becton Dickinson, New Jersey, USA). Sensitivity, specificity, positive and negative predictive values were calculated for detection of CIN 2+ histology for urine and for cervico-vaginal

self-collected specimens. Agreement between the sample collection methods was assessed by Cohen’s kappa statistic (κ) and percentage agreement.

Conclusion

Between Nov 2016 and Aug 2018, a total of 315 women ages 25–65 years (median age=36) had valid HPV testing results on all 3 sample collections and on cervical histology. Most women were either uninsured (41%) or had Medicaid/Medicare (21%). A third (36%) were privately insured, with half (48%) self-identifying as White, and 29% as Black. The Assay performance in each of the sample types is summarized in Table 1. The overall concordance among the three tests was 70.0% (κ =0.5, p < .0001). There was 74.6% agreement the urine and physician tests (κ = 0.44, p < .0001), 83.6% agreement between self-collected urine and cervico-vaginal tests (κ = 0.7, p < .0001), and 74.5% agreement between cervico-vaginal and physician tests (κ = 0.45, p < .0001).

TABLE 1	Sensitivity	Specificity	PPV	NPV
Provider-collected	89.2%	50.7%	46.4%	90.8%
Cervico-Vaginal self-collected	89.2%	29.6%	37.8%	85.1%
Urine self-collected	93.1%	27.7%	38.2%	89.4%

References

Self-collected urine hrHPV testing had similar performance to self-collected cervico-vaginal HPV testing. The option of self-testing for hrHPV may improve cervical cancer screening for women with limited access to healthcare.

00482

COMPARISON OF A DNA METHYLATION CLASSIFIER WITH HPV16/18 GENOTYPING AND REPEAT CYTOLOGY TRIAGE FOR DETECTION OF CIN2+ IN HPV POSITIVE WOMEN WITH ASC-US INDEX CYTOLOGY

16. Methylation

A.T. Lorincz [1], G.I. Sanchez [2], B. Nedjai [1], M.C. Agudelo [2], J.D. Villa [3], M.C. Kelly [3], A.R. Brentnall [1], J. Cuzick [1], T.A. Ramirez [2]

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Background / Objectives

HPV16/18 genotyping and cytology are proposed as triage tests for proper referral to colposcopy of HPV positive women. We compared HPV16/18 genotyping and repeat cytology with the new DNA methylation test S5 (based on weighted methylation measurements of gene regions, including human gene EPB4IL3, and late gene regions of HPV16, HPV18, HPV31 and HPV33) in HPV positive women with ASC-US index cytology.

Results

167 women with expert confirmed CIN2+ and 167 age- and date of diagnosis matched controls (<CIN2) were identified in a cohort with ASC-US index cytology, followed-up in routine clinical services for 24 months. Archived HPV positive samples from the recruitment visit were HPV genotyped and tested for methylation, blinded to cytology, histology and initial HPV results. The sensitivity and specificity of cytology was determined using the worst cytology repeated at 6 or 12 months after the ASC-US index cytology, at a threshold of ASC-US or above. The performance of HPV genotyping and S5 were determined from baseline specimens. At our standard cut-off of 0.8 (previously validated for a screening population) for the continuous S5 classifier, and also in a post-hoc analysis with the S5 cut-off at the upper quintile

(3.1) of the control group, the S5 values were used to dichotomize methylation results as positives and negatives.

Conclusion

S5 median methylation level was 1.06 in histology Negative (n=125), 1.42 in CIN1 (n=42), 3.72 in CIN2 (n=123), 8.0 in CIN3 (n=40) and 10.84 in cancer (n=4) (Cuzick test for trend $\chi 2 = 42.6$, $p < 0.001$). Sensitivity of HPV16/18 genotyping was 52% (95%CI: 43-61%) and of cytology was 28% (95%CI: 17-39%). Performance of S5 at the pre-defined screening cut-off of 0.8 had poor specificity (sensitivity 84%, 95%CI: 79-89%; specificity 34%, 95%CI: 27-44%) which was significantly ($p < 0.001$) lower than the specificity of HPV16/18 (64%, 95%CI: 56-71%) or cytology (79%, 95%CI: 73-84%). In contrast at a cut-off 3.1 sensitivity of S5 was 58% (95%CI: 50-66%), similar to the sensitivity (52%) of HPV16/18 testing and much better than the sensitivity of cytology. Also, the specificity of S5 at this cut-off was 74% (95%CI: 67-80%), significantly higher than for HPV16/18 testing ($p=0.03$) and similar to cytology ($p = 0.38$). The AUC of the continuous S5 classifier (0.71, 95%CI: 0.65-0.77) was significantly higher (Delong test <0.001) than the AUC of either HPV16/18 (AUC: 0.58, 95%CI: 0.52-0.64), cytology (AUC: 0.54, 95%CI: 0.48-0.60) separately, or both combined (0.57, 95%CI: 0.51-0.63).

References

S5 was superior to cytology, HPV16/18 genotyping or their combination, for detecting CIN2+ in HPV+ women with an ASC-US index cytology.

FC 17. Methylation 2

00128

METHYLATION IN HPV16 E2 BINDING SITES 3/4 IS INDEPENDENT OF GLOBAL HOST GENOME METHYLATION AND RELATED TO SURVIVAL IN A COHORT OF OPSCC PATIENTS

16. Methylation

M.S. Kalteis [1], M. Reuschenbach [1], S. Vinokurova [2], U. Keilholz [3], A. Zakarneh [4], I. Tinhofer [5], M. Von Knebel Doeberitz [1], E.S. Prigge [1]

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Background / Objectives

The HPV16 upstream regulatory region (URR) undergoes shifts in methylation during HPV-induced carcinogenesis. Four binding sites for E2 (E2BS), a key regulatory protein of HPV E6/E7 oncogene expression, are located there. Methylation of these sites prevents E2 from fulfilling its function and thereby in turn promotes the overexpression of the E6 and E7 oncogenes which is the key step in HPV-induced tumorigenesis.

We have shown previously that shifts in E2BS methylation occur during metastasis formation in oropharyngeal squamous cell carcinomas (OPSCC). We hypothesized that these changes in the HPV genome's epigenetic signature occur in a specific manner independent of global host genome methylation status and could be indicative of a distinct functional role of E2BS methylation in HPV-driven OPSCC. Additionally, changes in E2BS methylation might be related to clinical outcome.

Results

Formalin-fixed, paraffin-embedded tissue sectioned from 67 simultaneously HR-HPV 16-driven, i.e. HPV16 DNA+ and $p16^{INK4a}$+, tumors (42 OPSCC primaries with 25 matched lymph-node metastases) was obtained from a German OPSCC cohort along with corresponding clinical data. Bisulfite-converted DNA was analyzed for methylation ratios in 4 CpGs in E2BS3 and 4 as well as 3 CpGs in the LINE1 retrotransposon by pyrosequencing. Cut-offs for low/high methylation were

established using cluster analysis. Kaplan-Meier-curves and log-rank-tests were utilized to examine overall and progression-free survival (OS, PFS).

Conclusion

Lower methylation levels could be observed in all E2BS CpGs in lymph node metastases when compared to their matched OPSCC primaries, with this difference reaching statistical significance for the CpG at position 43 in E2BS3 (p=.02). There was no significant difference detectable in LINE1 methylation as a marker of global genome methylation between primary tumors and their corresponding metastases. High E2BS methylation in primary tumors (> 52%) was associated with both reduced OS and PFS.

References

Shifts in E2BS methylation in OPSCC occur in specific patterns that are not associated with global host genome methylation as assessed in LINE1 retrotransposons in our cohort of HPV-driven primary tumors and associated metastases. Furthermore, these changes in epigenetic markup were found to be associated with clinical outcome parameters. These findings suggest that distinct HPV16 URR methylation patterns play a functional role during OPSCC progression and have an impact on patient survival.

00164

DETECTION OF HYPERMETHYLATED GENES AS MARKERS FOR CERVICAL SCREENING IN WOMEN LIVING WITH HIV

16. Methylation

W.W. Kremer [1], M. Van Zummeren [1], P.W. Novianti [1], K.L. Richter [2], W. Verlaat [1], P. Snijders [1], D. Heideman [1], R. Steenbergen [1], G. Dreyer [3], C. Meijer [4]

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Background / Objectives

To evaluate the performance of hypermethylation analysis of *ASCL1*, *LHX8* and *ST6GALNAC5* in physician-taken cervical scrapes for detection of cervical cancer and cervical intraepithelial neoplasia (CIN) grade 3 in women living with HIV (WLHIV) in South Africa.

Results

Samples from a prospective observational cohort study were used for these analyses. Two cohorts were included: a cohort of WLHIV who were invited for cervical screening (n=321) and a gynaecologic outpatient cohort of women referred for evaluation of abnormal cytology or biopsy proven cervical cancer (n=108, 60% HIV seropositive). Cervical scrapes collected from all subjects were analysed for hypermethylation of *ASCL1*, *LHX8* and *ST6GALNAC5* by multiplex quantitative methylation specific PCR (qMSP). Histology endpoints were available for all study subjects.

Conclusion

Hypermethylation levels of *ASCL1*, *LHX8* and *ST6GALNAC5* increased with severity of cervical disease. The performance for detection of CIN3 or worse (CIN3+) as assessed by the area under the receiver operating characteristic (ROC) curves (AUC) was good for *ASCL1* and *LHX8* (AUC 0.79 and 0.81, respectively), and

moderate for *ST6GALNAC5* (AUC 0.71). At a threshold corresponding to 75% specificity, CIN3+ sensitivity was 72.1% for *ASCL1* and 73.8% for *LHX8* and all samples from women with cervical cancer scored positive for these two markers.

References

Hypermethylation analysis of *ASCL1* or *LHX8* in cervical scrape material of WLHIV detects all cervical carcinomas with an acceptable sensitivity and good specificity for CIN3+, warranting further exploration of these methylation markers as a stand-alone test for cervical screening in low-resource settings.

00207

Host-cell DNA methylation patterns during high-risk HPV-induced carcinogenesis reveal a heterogeneous nature of cervical pre-cancer

16. Methylation

G.B.A. Wisman [1], W. Verlaat [2], R.W. Van Leeuwen [1], P.W. Novianti [2], E. Schuuring [3], C.J.L.M. Meijer [2], A.G.J. Van Der Zee [1], P.J.F. Snijders [2], D.A.M. Heideman [2], R.D.M. Steenbergen [2]

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Background / Objectives

Cervical cancer development following a persistent infection with high-risk human papillomavirus (hrHPV) is driven by additional host-cell changes, such as altered DNA methylation. In previous studies, we have identified 12 methylated host genes associated with cervical cancer and pre-cancer (CIN2/3). This study systematically analyzed the onset and DNA methylation pattern of these genes during hrHPV-induced carcinogenesis using an longitudinal in vitro model of hrHPV-transformed cell lines (n=14) and hrHPV-positive cervical scrapings (n=113) covering various stages of cervical carcinogenesis.

Results

DNA methylation analysis was performed by quantitative methylation-specific PCR (qMSP) and relative qMSP values were used to analyze the data.

Conclusion

The majority of genes displayed a comparable DNA methylation pattern in both cell lines and clinical specimens. DNA methylation onset occurred at early or late immortal passage, and DNA methylation levels gradually increased towards tumorigenic cells. Subsequently, we defined a so-called cancer-like methylation-high pattern based on the DNA methylation levels observed in cervical scrapings from

women with cervical cancer. This cancer-like methylation-high pattern was observed in 72% (38/53) of CIN3 and 55% (11/20) of CIN2, whereas it was virtually absent in hrHPV-positive controls (1/26).

References

In conclusion, hrHPV-induced carcinogenesis is characterized by early onset of DNA methylation, typically occurring at the pre-tumorigenic stage and with highest DNA methylation levels at the cancer stage. Host-cell DNA methylation patterns in cervical scrapings from women with CIN2 and CIN3 are heterogeneous, with a subset displaying a cancer-like methylation-high pattern, suggestive for a higher cancer risk.

00247

HR-HPV INFECTION AND THE METHYLATION OF P16INK4A IN WOMEN WITH HSIL IN CERVIX BEFORE AND AFTER TREATMENT.

16. Methylation

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Background / Objectives

One of the mechanisms by which high-risk human papillomavirus (hr-HPV) alters DNA methylation profile is targeting the epigenetic mechanisms through enzymes (Anayannis, Schlecht, and Belbin 2015). The methylation of p16 was found as a risk factor for ASCUS/LSIL progression to HSIL (Lee and Lee, n.d.). However, there is a lack of research on follow-up outcome for women with HSIL with methylated p16 after treatment.

Results

In the pilot, longitudinal cohort study 25 women with the diagnosis of HSIL were included in Antônio Pedro University Hospital, Brazil in 2011-2012. Methylation-specific polymerase chain reaction was performed using cytology samples collected before surgery and 6 months after to evaluate methylation of the p16INK4a. Hr-HPV was detected using HPV E6/E7 mRNA in situ hybridization.

Conclusion

Hr-HPV infection was found in 72.0% (18/24) cases. Promoter methylation of p16INK4a occurred in 72.0 (18/25) cases, but significantly did not differ between hr-HPV positive (72.2%, 13/18) and negative (66.7%, 4/6) samples (OR 1.3, CI95% 0.2-9.5, p =1.0). Expression of p16INK4a was found in 52.0% (13/22) and commonly detected together with methylated p16INK4a (66.7%, 10/15), however, the result has

not reached statistical significance (p=0.4). The silencing of p16INK4a was not significantly associated with methylation status, even in HPV-infected samples in which the p16INK4a promoter was methylated (>0.05).

In CIN III group all but one had the persistence of the same or higher expressed promoter methylation of p16INK4a after surgery (Wilcoxon Sing rank test p=0.02). This trend was evident in total sample as well (Wilcoxon Sing rank test p=0.01), but was not related to immunohistochemically detected expression of p16INK4a in tissue (Mann-Whitney U p=0.9), hr-HPV diagnosis before (Mann-Whitney U p=0.3) and after surgery (Mann-Whitney U p=1.0), compromised margins (Mann-Whitney U p=0.4), grade of lesson (Kruskal Wallis Test p = 0.3).

References

Methylation of p16INK4a is the evident process in women with cervical lesions. The process is noticed even after surgery, thus more research is necessary to clarify it's clinical and pathophysiological significance.

References

Anayannis, Nicole V. J., Nicolas F. Schlecht, and Thomas J. Belbin. 2015. "Epigenetic Mechanisms of Human Papillomavirus–Associated Head and Neck Cancer." Archives of Pathology & Laboratory Medicine 139 (11): 1373–78. https://doi.org/10.5858/arpa.2014-0554-RA.

Lee, Hee, and Eun-Ju Lee. n.d. "HPV Infection and p16 Promoter Methylation as Predictors of ASC-US/LSIL Progression." Cancer Cytopathology 124 (1): 58–65. https://doi.org/10.1002/cncy.21615.

00360

SIX METHYLATION MARKERS, KNOWN AS GYNTECT ASSAY, SHOW A VERY GOOD PERFORMANCE IN A TRIAGE SETTING ON HPV POSITIVE WOMEN

16. Methylation

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Background / Objectives

HPV DNA testing as a primary screening marker is being implemented in several countries. Due to the high HPV prevalence in the screening population, effective triage strategies for HPV-positive cases are required. The aim of this study was to evaluate the performance of a methylation-specific real-time PCR assay (GynTect) comprising six marker regions as a triage test.

Results

In a retrospective, cross-sectional study with the colposcopy clinic of Jena University Hospital, cervical scrapes from 675 patients were analyzed using methylation specific PCR for 6 (ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671) promising DNA methylation marker regions, called GynTect markers. We correlated the GynTect results to histopathological findings.

Conclusion

The GynTect methylation markers show a 100% sensitivity for CIS and cancer scrapes (n=31), irrespective of suptype. 64.1% CIN3 were detected followed by 30% CIN2 and 12.5% CIN1, respectively. In the HPV positive, but biopsy proven “no CIN group”, of 19.2% GynTect are positively tested. In total, sensitivity and specificity for CIN3+ in this cohort was 72.4% and 85.2%, respectively.

In contrast, real negatives from routine screening (HPV negative and Pap I, n=545) show an extra ordinary low positivity rate for GynTect® of 1.1%.

References

The performance of the GynTect assay based on cervical scrapes from the colposcopy clinic in Jena provides good evidence for the usefulness of methylation markers to detect HPV-positive women with clinically relevant disease.

00362

A PANEL OF SIX DNA METHYLATION MARKERS, COMPRISING THE GYNTECT CERVICAL CANCER TRIAGE TEST, DISPLAY EXCELLENT SENSITIVITY FOR CERVICAL CARCINOMAS

16. Methylation

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Background / Objectives

A prerequisite for triage tests complementing HPV-based cervical cancer screening, which now is or is being established in several countries (USA, the Netherlands, Ireland, Australia, Germany), is to detect cervical cancer with high sensitivity at no loss of specificity. DNA methylation has been discussed by experts as an attractive option in that context, and with GynTect a molecular diagnostic test is available based on this class of markers. The aim of this study was to assess the methylation of the six markers comprising GynTect in cervical carcinomas using both, cervical tissue and cervical scrapes.

Results

DNA isolated from 155 cervical cancer tissues as well as scrapes from 79 cancer and 14 CIS/ACIS patients taken before surgical treatment at the university women's hospital in Jena were included in the study comprising squamous cell carcinomas as well as adeno carcinomas. Methylation of the six marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671 was assessed performing methylation-specific PCR on chemically treated DNA from the cancer samples.

Conclusion

All 155 cervical tissues showed methylation of at least two of the six markers, and each of the methylation markers was positive in at least 80% of the cervical cancer tissues. Similar results were obtained when analysing the methylation of the six markers using the GynTect assay for the cervical scrapes from cancer and CIS/ACIS patients.

References

The six DNA methylation markers can be detected in tissue as well as scrapes obtained from cervical cancer patients with different sensitivities, ranging from 95% to 100%. The CE IVD marked GynTect assay for cervical cancer triage that is based on detection of these six markers, allows detection of all CIS/ACIS and all cancer cases with 100% sensitivity. Detection rates are irrespective of the type of cancer, allowing the reliable detection of both, squamous cell carcinomas and adeno carcinomas.

00389

Is human papillomavirus DNA methylation an accurate diagnostic marker for detection of women with abnormalities at cervical cancer screening?: A Systematic Review and Meta-analysis.

16. Methylation

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Background / Objectives

The introduction of HPV DNA testing for primary cervical screening aims to improve sensitivity of the screening. It will also increase positive test results and the best policy for triage of HPV-positive women is still unclear. Viral DNA methylation has been proposed as a novel biomarker for triage with encouraging but conflicting results. We undertook a systematic review and meta-analysis of published literature to assess the correlation of HPV DNA methylation with disease grade for prediction of high-grade intraepithelial disease (CIN2+).

Results

We searched electronic databases MEDLINE, EMBASE and CENTRAL. Studies were eligible if HPV epigenome was analysed by any DNA methylation method, with corresponding cytology or histology results available. Data were pooled and meta-analysed using random effects models in STATA.

Conclusion

42 studies were eligible for inclusion. Increasing methylation of the HPV16 L1 gene showed the greatest association with increasing disease grade (Normal: 14% (95%CI 3-37); CIN1: 42% (95%CI 7-83); CIN2: 53% (95%CI 5.2-98); CIN3: 77% (95%CI 29-100); invasive cervical cancer (ICC): 77% (95%CI 55-95). Pooled methylation percentage was significantly higher in CIN2+ vs. CIN2- (72.8% (95%CI 49-92) vs.

44% (95%CI 16-74), p<0.0001). For bisulphite sequencing methods only, overall pooled estimated odds ratio (OR) (95%CI) for high methylation in the HPV16 L1 gene for CIN2+ vs. CIN2- was 2.15 (95%CI 0.82-5.6), I290.6%. For pyrosequencing methods, the highest OR was observed at CpG site L1 5602 (OR 36.8, 95%CI 8.8-153). Pooled sensitivity and specificity for diagnostic accuracy of HPV16 L1 methylation in detection of CIN2+ were 0.83 (95%CI 0.74-0.90) and 0.62 (95%CI 0.54-0.69) (AUC 0.75 (0.71-0.79), I290% (95%CI 81-100)).

References

HPV16 L1 gene methylation levels correlate with increasing grade of CIN, with significantly higher methylations levels observed in CIN2+ vs. CIN2-. Sensitivity and specificity are highly variable by CpG site and estimates vary significantly between studies. Our results suggest that HPV methylation could be an accurate marker of high grade disease, but the genes and CpG sites most discriminatory must still be identified for clinical practice, and the role of methylation as a diagnostic test to triage HPV-positive women warrants further investigation.

00391

HOST DNA METHYLATION PANEL VS CYTOLOGY FOR HR-HPV POSITIVE CASES TRIAGE

16. Methylation

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Background / Objectives

There is strong agreement in the scientific community regarding the superiority of high risk (hr) HPV testing compared to cytology in cervical cancer primary screening. Limited specificity of hr-HPV testing, however, requires new biomarkers for the triage of HPV-positive cases, in order to avoid overtreatment and excessive referral to colposcopy. In fact, this lack of specificity of hr-HPV testing leads to a risk of overloading colposcopy. DNA methylation (viral and host) has been proposed as a promising strategy for triaging HPV-positive women in order to overcome this problem. This work aims to evaluate the potential of a host DNA methylation panel (Hansel et al., 2014; Schmitz et al., 2017) as an alternative to reflex cytology in triage.

Results

A maximum of 100 consecutive PreservCyt samples positive for hr-HPV testing (Roche COBAS® HPV) were selected from routine screening at LAP Unilabs Porto. For all these samples, cytology was performed (ASCUS and above considered cytology positive). Follow-up histology data generated for each patient will be included if available. Information regarding the evaluation sample, patient information and data regarding other clinical samples results relating 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 were anonymized.

All DNA methylation panel testing was performed in the COBAS Z480 analyzer (component of the COBAS4800® system, which was used for HPV screening).

According to previous data generated for this host DNA methylation panel, we will assume a 100% sensitivity for cervical invasive carcinomas. To assume this performance in this study we will include at least ten cervical carcinomas in parallel to confirm it.

References

Data has been collected and is not fully available at the present time. We expect a lower positivity rate in the methylation panel, leading to increased specificity – without losing sensitivity - compared to cytology and therefore, with this approach reduce substantially the required number of colposcopy procedures. Comparing the costs of both approaches a cost effectiveness analysis could be performed and evaluate the viability of methylation analysis.

References

Hansel, A., Steinbach, D., Greinke, C., Schmitz, M., Eiselt, J., Scheungraber, C., … Dürst, M. (2014). A promising DNA methylation signature for the triage of high-risk human papillomavirus DNA-positive women. PLoS ONE, 9(3). http://doi.org/10.1371/journal.pone.0091905

00441

COMPARISON OF TWO METHYLATION BASED DIAGNOSTIC ASSAYS ON A COHORT OF CA 130 HPV POSITIVE CERVICAL SCRAPES: GYNTECT AND QIASURE

16. Methylation

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Background / Objectives

HPV DNA testing as a primary screening marker is being implemented in several countries. Due to the high HPV prevalence in the screening population, effective triage strategies for HPV-positive cases are required. Methylation markers are presently discussed as a suitable tool for triaging HPV positive women. We compared the two assays GynTect and QIAsure.

Results

In a retrospective setting 130 samples from the colposcopy clinic of the university hospital in Jena, all tested HPV positive, were tested with GynTect, comprising 6 (ASTN1, DLX1, ITGA4, RXFP3, SOX17, ZNF671) different methylation and QIAsure, comprising 2 (FAM19A4, mir124) methylation markers. The cohort comprises 7 cervical cancer scrapes, 49 CIN3, 8 CIN2 and 5 CIN1 and 59 no CIN samples. In addition, 10 HPV negative Pap I samples were tested.

References

Analyses are still ongoing and should be completed for the meeting. We expect a similar detection rate regarding the cancer cases and CIN2 and CIN3 samples. Looking at the no CIN group, higher detection rates are published for QIAsure assay (IFU) as compared to the GynTect assay. A direct comparison between both methylation marker panels has never been done so far.

00443

PERFORMANCE OF GYNTECT®, A DNA METHYLATION MARKER PANEL-BASED DIAGNOSTIC TEST, ON A WIDELY USED PCR DIAGNOSTICS PLATFORM

16. Methylation

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Background / Objectives

A change of the current screening algorithms to an HPV-based screening setting is discussed in several countries due to higher sensitivity of HPV testing compared to cytology. Reliable triage methods, ideally performed from the sample obtained for screening are, however, essential in such a setting to avoid overtreatment and higher screening costs. Specific DNA methylation patterns may provide a suitable triage tool, and they can be detected using molecular tests that do not require specific equipment.

Results

Cervical scrapes collected in PreservCyt® solution from women with cervical cancer (35 cases), CIN 1-3 (120 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) comparing the 7500 QPCR system (Life Technologies; Thermo Science) routinely used for the assay with the COBAS Z480 QPCR system (Roche Diagnostics).

References

Analyses are still ongoing and will be finished before the meeting. In a smaller evaluation set of samples (n=87), the 100% sensitivity for cancer cases (n=5) could be confirmed and a quite good specificity (CIN3+) of 74% was achieved. False positive rate in PapI, HPV negative samples was 2% (1 out of 43). Overall, the concordance between the two systems in this subset was 90%.

The newly established PCR protocol for the COBAS Z480 QPCR system seems to allow the performance of the GynTect assay with an accuracy comparable to the

original protocol run on the 7500 QPCR system. Analysis of the whole dataset will be presented at the meeting.

00468

GENOME-WIDE DNA METHYLATION PROFILING IDENTIFIES TWO NOVEL METHYLATED GENES TO PREDICT PROGRESSION OF CERVICAL INTRAEPITHELIAL NEOPLASIA

16. Methylation

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Background / Objectives

DNA methylation analysis is a promising approach to complement information on human papillomavirus genotyping in discriminating risk of progression along the continuum of cervical intraepithelial neoplasia (CIN) grades and cervical cancer. We used a pan-epigenomic approach to identify new methylation markers that discriminate among CIN grades, evaluated the correlation between methylation levels and lesion grade, and developed algorithms for risk prediction.

Results

The Methylation Analysis Revealing Key Epigenetic Regulation (MARKER) study comprised 186 physician-collected cervical samples (54 normal, 50 CIN1, 40 CIN2, and 42 CIN3) randomly selected from 643 women referred for evaluation of abnormal cytology at a single-center colposcopy clinic. Extracted DNA was subjected to Illumina Infinium EPIC array analysis. Raw data were analyzed using the ChAMP package in R; applying normalization with BMIQ and batch effect correction for technical replication with ComBat. We implemented the LIMMA package to calculate the p-value for differential methylation using a multiple linear regression model. We performed Spearman correlation analysis (Hmisc package) to determine whether DNA methylation changes correlate with progression. We assessed CpG sites whose state of methylation correlates with lesion grade by generating methylation index cutoff values and a weighted DNA methylation score, comparing normal to CIN3. Methylation markers were assessed via receiver-operating characteristic (ROC) curves for sensitivity and specificity as a function of methylation. Validation of the

identified genes was performed using an independent dataset of women with cervical cancer (GSE68339, n=270).

Conclusion

Our analyses revealed 7715 CpG sites whose DNA methylation level correlated with progression (from normal to CIN1, CIN2, and CIN3). There was a significant trend of increased methylation with disease grade. We identified a bigenic (hyaluronan synthase 1, HAS1 and ATPase phospholipid transporting 10A, ATP10A) methylation marker set; r=0.55, p<0.0001. ROC curve analysis demonstrated that the sensitivity and specificity were both 1.00 for detection of cancer, with independent validation revealing a significant positive correlation (r=0.88, p<0.0001).

References

Genome-wide DNA methylation profiling identified a potentially useful 2-gene methylation cancer prognostic marker. Further exploration of these methylated host genes to improve risk stratification in cervical screening is warranted.

00472

DNA METHYLATION TEST TO DETECT CERVICAL PRE-CANCER IN SELF-COLLECTED VAGINAL AND URINE SPECIMENS.

16. Methylation

B. Nedjai [1], M. Kleeman [1], C. Reuter [1], T. Hollingworth [1], L. Cadman [1], J. Austin [1], D. Patel [1], A. Parberry [2], L. Ashdown-Barr [1], J. Cuzick [1], L. Lorincz [1]

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Background / Objectives

Implementation of HPV testing as a primary screen is becoming the norm worldwide. HPV testing is a very sensitive method but not sufficiently specific, thus the choice of an appropriate triage strategy for hrHPV positive women is key. Clinician-taken samples are the gold standard but self-sampling may be a useful alternative for women unable or unwilling to undergo examination. Collection of urine samples offers another simple option, although the sensitivity of detecting CIN2+ by HPV DNA testing of urine is slightly lower than clinician-taken cervical samples (88.3% vs 94.5%; Cuzick et al., 2017). We developed a triage classifier (S5) for the detection of CIN2+, based on DNA methylation of HPV16, HPV18, HPV31 and HPV33, combined with the human gene EPB41L3 (Brentnall et al., 2015). Our goal was to assess the performance of S5 for detecting CIN2+ using both self-collected vaginal samples and urine.

Results

Women attending the colposcopy clinic at The Royal London Hospital as a consequence of abnormal screening cytology and/or a positive HPV result were recruited as part of the ‘Self-sampling for vaginal HPV: Predictors 5.1’ study. 503 women provided a first-flow urine sample using the Colli-Pee™ device with UCM storage buffer, of which 300 women provided self-collected vaginal samples using a flocked swab (Copan) re-suspended in PreservCyt. DNA was extracted and bisulfite converted, followed by pyrosequencing for S5. Average methylation was calculated for each component biomarker and the S5 score calculated.

Conclusion

S5 showed a highly significant separation between <CIN2 and CIN2+ for both urine and vaginal self-samples ($p<0.0001$). The area under the ROC curve was 0.73 (95%CI: 0.67 to 0.78, $p<0.0001$) for urine samples and 0.74 (95%CI: 0.67 to 0.81, $p<0.0001$) for vaginal self-samples. At the S5 pre-defined cut-off (0.8) sensitivity for CIN2+ in urine samples was 66% with specificity 72%, while for vaginal self-samples they were 71% and 68% respectively.

References

Our study in a colposcopy clinic shows that S5 is promising for testing of self-collected urine and vaginal samples to correctly identify most women with CIN2+. Self-sampling can have a big benefit for women in low and middle-income countries with limited access to effective screening and also for non-attendees in high income countries.

References

Cuzick J et al. 2017; Cancer Epidemiol Biomark Prev 26:1053. Brentnall A et al. 2015; Cancer Biomark 15:669.

00486

VALIDATION OF A DNA METHYLATION CLASSIFIER FOR PREDICTION OF CERVICAL PRE-CANCER IN THE MEXICAN FRIDA POPULATION-BASED HPV SCREENING STUDY

16. Methylation

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Background / Objectives

DNA methylation plays an important role in carcinogenesis and can be the basis of diagnostic and prognostic assays. We evaluated the clinical performance of the S5 DNA methylation score developed in the UK, combining methylation levels of HPV16, HPV18, HPV31, HPV33 and the tumour suppressor gene EPB41L3, as a predictive classifier of cervical intraepithelial neoplasia grade 2 or higher (CIN2+) in hrHPV-positive Mexican women.

Results

A nested case–control analysis from within the large population-based FRIDA screening cohort (ClinicalTrials.gov NCT02510027), focusing on hrHPV-positive women aged 30 to 64 years, with either positive cytology or HPV genotypes 16 or 18. Cases (CIN2+, n=79) were age-matched to controls (n=237) without CIN2+. DNA from exfoliated cervical cells was extracted, bisulfite converted, and amplified for S5 targets; methylation was quantified at specific CpG sites by pyrosequencing. The sensitivity, specificity, and predictive values of the S5 classifier were evaluated. A new proposed S5 cutoff of 3.75 for Mexico was selected by receiver operating characteristic (ROC) curve analyses.

Conclusion

S5 separated CIN2+ from CIN1/normal with an area under the ROC curve (AUC) of 0.75 (95% confidence interval: 0.69-0.82) while the AUC for CIN3+ was 0.81 (95%CI: 0.74-0.89). All 3 invasive cancers were detected and sensitivity for CIN2+ was 64.0% (95% CI 50.4–72.7%), with a specificity of 73.0% (95% CI 66.9–78.5%) and positive predictive value of 43.4% (95%CI: 34.0–53.0%). In comparison, the sensitivity and specificity of HPV16/18 genotyping was 67.1% (95% CI: 55.6-77.3%), and 26.6% (95% CI 21.1-32.7%), respectively, while PPV was 23.3% (95%CI: 18.0-29.4%). The S5 sensitivity and specificity for CIN3+ were 70.3% (95% CI 57.6-81.8) and 76.6% (95% CI 70.7-81.9 %), respectively

References

S5 may be useful as a new triage test for hrHPV-positive Mexican women with CIN2+ requiring referral for colposcopy. Our study confirms an earlier validation in a screening study of European women. S5 is a quantitative assay and various cut-offs can be selected and validated to suit different countries and test settings.

FC 18. Vaccines 5

00187

WHAT IS THE DIFFERENCE IN RISK BETWEEN UNVACCINATED AND VACCINATED WOMEN AGAINST HUMAN PAPILLOMAVIRUS

09. HPV screening

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Background / Objectives

Internationally, Human Papillomavirus (HPV) vaccination uptake in young girls is 30-80%. Will it be reasonable to screen – for cervical cancer - vaccinated and unvaccinated women alike? Next to feasibility issues, this depends on the difference in cervical cancer risk between the two groups. The risk in unvaccinated women will be depend on herd immunity, and thus on vaccination coverage and time since vaccination started. How much herd immunity is expected and when?

Results

We compared three HPV transmission models as part of the Cancer Intervention and Surveillance Modeling Network (CISNET) on predicted HPV16/18 infection incidence reduction and resulting herd immunity effect, assuming vaccination with 60% coverage. Herd immunity effect is defined as the cervical cancer incidence reduction in non-vaccinated women relative to vaccinated women (100% means cervical cancer risk is equal in unvaccinated and vaccinated women).

Conclusion

In the steady state, HPV16 incidence reduction was predicted to be 80, 73 and 65% in all women for the three independent models. This was reached after 70, 69 and 37

years respectively. For HPV18 the results were similar. The corresponding herd immunity effects were 56, 32 and 17% respectively.

References

Although herd immunity is important for decisions concerning cervical cancer screening, there seems to be uncertainty about its expected magnitude and the rate at which it will develop. Monitoring of the HPV prevalence, especially in unvaccinated women, in various populations, combined with comparative modeling, will add to the understanding of the dynamics of herd immunity and improve further projections.

00139

DECLINES IN ANOGENITAL WARTS DIAGNOSES SINCE THE CHANGE IN 2012 TO USE THE QUADRIVALENT HPV VACCINE IN ENGLAND: DATA TO END 2017

29. Genital warts

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Background / Objectives

In 2008, a national human papillomavirus (HPV) vaccination programme to prevent cervical cancer was introduced in England using the bivalent vaccine (HPV types 16 and 18 only). In 2012, the programme changed to offer the quadrivalent vaccine that additionally protects against the two HPV types that cause the majority of anogenital warts (AGW; HPV6 and 11). Coverage for the vaccination programme has been high, with over 85% of routine cohorts (12-13 year olds) completing the recommended schedule. We present data reporting AGW diagnoses in specialist Sexual Health Clinics (SHC) in England to the end of 2017, including diagnoses up to 17 years of age among birth cohorts offered routine vaccination with the quadrivalent vaccine.

Results

Data were obtained from the GUMCAD STI Surveillance System (GUMCAD), submitted by SHC, for years 2009-2017. This surveillance system includes data on all attendances and diagnoses at SHC in England. Records coded as first episode AGW for females, heterosexual males, and men who have sex with men (MSM) aged 15-17 years old were extracted. The incidence of AGW diagnoses per 100,000 population was calculated using national population estimates by sex and age, and the percentage of men who have sex with men (based on data published in the Natsal-3 study of sexual attitudes and lifestyles in the UK). Percent declines in the rates of AGW diagnoses were calculated between 2014 and 2017, during which most females aged 15-17 years would have been offered the quadrivalent vaccine. Poisson regression was used to test for trends over time for each sub-group.

Conclusion

Between 2014 and 2017, there were substantial declines in the incidence of AGW diagnoses in SHC among young females aged 15-17 years, from 257.5 per 100,000 population in 2014 to 45.7 per 100,000 population in 2017 (82.3% decline, p-value for trend <0.001). Declines in the incidence of AGW diagnoses were also seen among same aged heterosexual males (67.7% lower in 2017 than 2014 in 15-17 year old heterosexual males, p-value for trend <0.001). Reductions in AGW diagnoses in MSM aged 15-17 years were less clear (decreased by 13.6%, from 129.9 in 2014 to 112.2 in 2017 per 100,000 population, p-value for trend 0.219).

References

Substantial declines in AGW diagnoses have been seen in young females who would have been offered the quadrivalent vaccine as part of the national HPV vaccination programme, as well as among same aged heterosexual males, strongly suggesting a herd protective effect. Weaker declines were also observed in young MSM. Surveillance plans are in place to continue to monitor AGW diagnoses to evaluate the impact of both female and targeted MSM HPV vaccination.

00328

EXTINCTION OF HPV 6 AND GENITAL WARTS IN A POPULATION WITH SUBOPTIMAL HPV VACCINE COVERAGE

29. Genital warts

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Background / Objectives

The 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 (via herd protection).Howeover, it is uncertain what level of vaccine coverage we require for such cohort effects.

Results

WOLVES (Wolfsburg HPV epidemiological study) invited to participate all women born 1983/84, 1988/89 and 1993/94 with first residency in Wolfsburg. The participants received annual visits with HC2-HPV testing and genotyping with SPF-10 PCR of all positive and 10% of HC2 negative samples.

Conclusion

Between Oct 2009 and Jan 2018 2477 women were included. The HPV vaccination coverage rate rose from 6.1% to 18.4% among 26 years old women. The corresponding rates for 21 years old women increased from 23.7% to 48.7%. Simultaneously the life risk to suffer from at least one episode of genital warts before age 27 dropped from 4.7% to 2.5%. The 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 and from 2.0% to 0.0% among 21 years old women in our population.

References

WOLVES is the first real- life study population (not exclusive data base analysis) to look at the impact of HPV vaccination under other on genital warts prevalence and HPV 6 incidence in Germany. We observed the unexpected decline of genital warts

and complete disappearance of HPV 6 in a population with suboptimal HPV vaccine coverage.

00148

PUBLIC HEALTH AND ECONOMIC IMPACT OF HPV VACCINATION IN THE PORTUGUESE NATIONAL IMMUNIZATION PROGRAM

36. Public health

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Background / Objectives

In Portugal, HPV vaccination was included into the National Immunization Program (NIP) for 13-years girls in 2008 using the 4-valent HPV (4vHPV) vaccine. In 2017 the 4vHPV vaccine was replaced by 9-valent HPV (9vHPV) vaccine and the age for vaccination was anticipated for 10 years. The aims are: (i) to assess the public health and economic impact of the first 10 years of HPV vaccination in the NIP; (ii) to estimate the impact for the upcoming years. An additional scenario considering gender neutral vaccination with 9vHPV vaccine was run.

Results

A published HPV disease transmission dynamic model accounting for herd protection effects with a lifetime horizon (100 years) has been adapted and calibrated for Portugal. The model considered the occurrence of cervical intraepithelial neoplasia (CIN), cervical, vaginal, vulvar and anal cancers; and also penile and oropharyngeal cancers. Demographic inputs were obtained from Statistics Portugal and annual all-cause mortality rates were extracted from the Portuguese Mortality table 2014-2016. Costs were taken from Santana et al[1] study and Decree 207/2017.

Conclusion

The first 10 years of HPV vaccination in NIP resulted in the reduction of CIN 1, CIN 2/3 and genital warts incidence (11,465 events avoided) and savings of €4,914,150. The maintenance of the current program for next 90 years will result in the avoidance of 1,437,091 events (e.g., cervical, vaginal, vulvar, anal, oropharyngeal cancers, CIN

1, CIN 2/3 and genital warts) and potential savings of €1,148,292,188. The implementation of universal vaccination program using the 9vHPV vaccine (boys and girls aged 10 years) will significantly reduce the clinical and economic burden resulting in 1,788,610 events avoided and savings of €1,329,629,689) compared to cervical cancer screening only.

References

The first 10 years of HPV vaccine in NIP resulted in additional benefits for the Portuguese Public Health, which become greater as time progresses. Notwithstanding, the reduction of the burden and expenditure related to HPV-related diseases can be amplified by the implementation of a 9vHPV universal vaccination program.

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00273

TYPE-SPECIFIC HUMAN PAPILLOMAVIRUS PREVALENCE IN THE NORTH OF MEXICO, A 10 YEAR STUDY AND RELATION WITH HPV VACCINE COVERAGE

36. Public health

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Background / Objectives

Cervical cancer is the second cause of death worldwide, and still the leading cause of death from cancer in Mexico. Human Papillomavirus(HPV) is the main cause of cervical cancer. Prevalence of cervical HPV infection varies worldwide and data regarding HPV prevalence and genotypes in Mexico is still limited. Detection and genotyping of HPV is an essential tool for screening, diagnosis, and management of HPV-related cervical cancer and its precursor lesions. The purpose of this study was to find data on prevalences of HPV genotypes, and prevalence of infections with multiple HPV genotypes in our population to improve specific prevention and management activities.

Results

This research was done at IMIGO (Instituto Mexicano de Infectologia, Ginecoologia y Obstericia) in Monterrey Mexico, with the database from our patiens from year 2008 to 2018. The kits used for typification were Innolippa HPV Genotyping Extra I and II, Linear Array HPV Genotyping Test, Seeplex HPV 18 ASE DPO and Abbot Real Time all processed at the Molecular Diagnosis Unit of the Hospital Universitario U.A.N.L. in Monterrey Mexico. The inclusion criteria were as follows: women and men with a history of sexual activity with risk of HPV infection, patients with positive HPV infection, and patients requesting an HPV test. We obtained 816 samples: 524 positive and 292 negative for HPV infection.

Conclusion

In our 524 positive samples, a total of 957 different HPV viruses were isolated.The most prevalent HR HPV serotypes found are HPV-16 (8.78%), HPV-51 (8.67%), HPV-52 (7.84%), HPV-33 (7.75%), HPV-31(5.54%), and LR most prevalent

was HPV-6 (6.79%). Overall 56.2% are HR HPVs, 11.7% probably HR, 28.5% are LR and only 3.55% of the virus could´t be typified by our test kits. In the 524 positives samples we found that 56% had mono-infections and 44% had multiple infections as high in some cases as 8 diferent serotypes in one patient(. Specifically 26.5% had multiple HPV infection with 2 serotypes, 9.92% with 3 serotypes, 4.01% with 4 serotypes, 2.1% with 5 serotypes and 1.15% with 6 serotypes .

References

HPV prevalence in our population was high 64%, with more than 56% having HR serotypes and up to 44% having multiple infections. We found that from the top 6 most prevalent virus found in our study were 5 HR and 1 LR and from those 5 are included in the HPV9 vaccine, confirming a strategic increased benefit over the other vaccines available in reducing the risk of cancers caused by HPV in our country.

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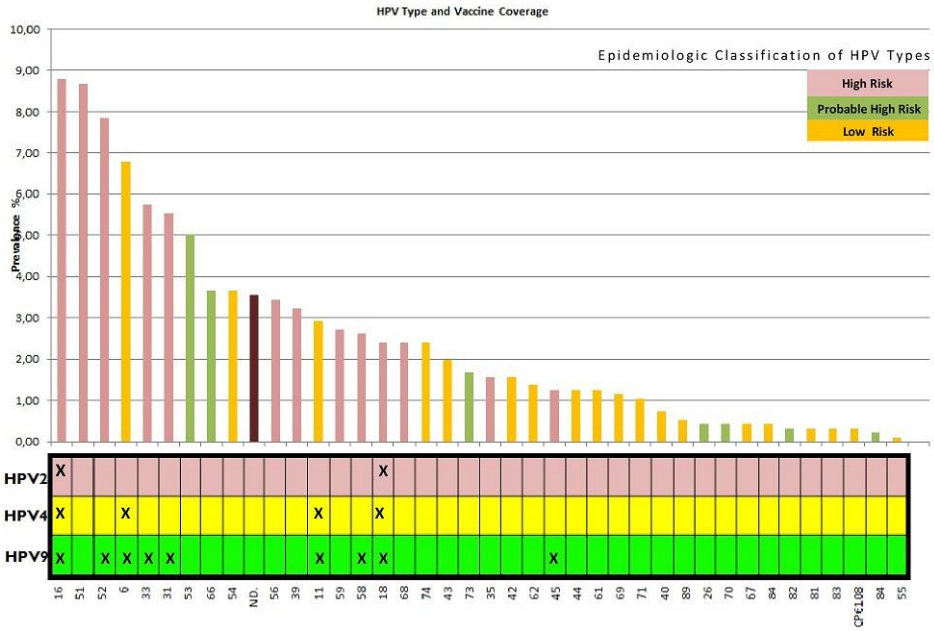
HPV Type and Vaccine Coverage
Epidemiologic Classification of HPV Types
High Risk
Probable High Risk
Low Risk
Prevalence %
10,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
HPV2
HPV4
HPV9
16 51 52 6 33 31 53 66 54 ND. 56 39 11 59 58 18 68 74 43 73 35 42 62 45 44 61 69 71 40 89 26 70 67 84 82 81 83 CP6108 84 55

00274

HPV SEROPREVALENCE AND GENITAL HPV INFECTIONS IN A COHORT OF YOUNG WOMEN IN THE NETHERLANDS SEVEN YEARS POST-VACCINATION

36. Public health

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Background / Objectives

HPV vaccination is effective against persistent infections and induces a robust serological response. However, many questions remain about vaccine induced or naturally derived antibodies and their role in protection. Therefore, we aim to describe the immunogenicity of the bivalent vaccine in a population-based setting up to seven years (7y) post-vaccination and to explore the longitudinal relation between HPV DNA presence and humoral response against HPV among vaccinated.

Results

A prospective cohort study including 1151 girls (birth cohort 1993) who were eligible for the catch up campaign in 2009 was performed in the Netherlands. One month prior to vaccination and each consecutive year post vaccination a vaginal self-swab, a serum sample, and a questionnaire were collected. A VLP-based multiplex immunoassay (MIA) was used to measure type specific HPV antibodies against HPV16, 18, 31, 33, 45, 52 and 58. The HPV-DEIA-LiPA25 could detect twenty-five HPV DNA genotypes including the high-risk types from the MIA. Type specific geometric mean concentrations (GMC) of serum IgG and seroprevalences were calculated (cut-offs described in (1)). We explored the association between IgG and incident infection in the subsequent round in a multilevel linear model.

Conclusion

1038 participants with baseline measurement were included (71.7% vaccinated). GMCs against vaccine types HPV16/18 peaked after vaccination and remained high up to 7y post-vaccination (seroprevalence 99-100% in vaccinated vs. 10-18% in unvaccinated 7y post-vaccination). GMCs against cross protective types HPV31/45

were lower, but still significantly higher than in unvaccinated individuals (GMC ratio 7y post-vaccination HPV31: 13.7, 95%C.I. 11.0-17.1, HPV45: 9.8, 95%C.I. 7.9-12.2). The association between IgG and incident infection in the subsequent round in vaccinated showed higher GMCs in uninfected participants compared to those with an HPV infection for HPV16 (GMC ratio 2.1, 95% C.I. 0.5-9.7), HPV31 (1.3, 95%C.I. 0.6-3.0), HPV45 (1.8, 95%C.I. 0.4-8.1). However, the opposite was seen for HPV18 (0.6 95%C.I. 0.1-3.1).

References

Serum IgG antibody responses against vaccine types remain high up to 7y post-vaccination in a population based setting. No significant association was found between type specific IgG and incident infection in the subsequent round , although vaccinated individuals with break-through infections showed slightly lower antibody levels one year before the infection. Still, this effect was not observed for all HPV types, providing no clear indication for the role of serum antibodies in protection against infection. The association between antibodies and infection at other time points will be explored further.

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00453

IMPACT OF A SINGLE-COHORT HPV VACCINATION STRATEGY WITH QUADRIVALENT VACCINE IN NORTHEAST SPAIN: POPULATION-BASED ANALYSIS OF GENITAL WARTS IN MEN AND WOMEN

36. Public health

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Background / Objectives

Catalonia, a region of 7.5 million inhabitants in northeast Spain, started in 2008 a school-based, single-cohort HPV vaccination programme targeting 11-year-old girls and offering the quadrivalent HPV vaccine free of charge, except for the year 2010 when the bivalent HPV vaccine was used. Throughout the observation period coverages have been steadily over 80%. Vaccine coverage for non-eligible cohorts is estimated to be very low. We aimed to estimate the population impact of a female single-cohort HPV vaccination programme through analysis of trends in genital warts (GW) diagnoses in a high coverage scenario.

Results

Data were obtained from the Information System for Research in Primary Care (SIDIAP), a population-based database of anonymised electronic health records that includes information of approximately 5.6 million patients (74% of Catalonian population). We retrieved all records of new GW diagnosed between 2009-2016 and for comparison purposes all new diagnoses of genital herpes and other STI were also collected. Annual incidence rates were calculated stratified by age and sex using joinpoint regression to estimate trends and annual percentage changes (APC). The first vaccinated cohort (girls born in 1997) turned 19 in 2016, the end of the study period.

Conclusion

GW incidence among women aged 16 to 19 years decreased a 61% from 2012 to 2016 (APC -19.4; 95% CI: -30.0%- -7.3%). In contrast, the incidence of genital herpes in same-aged women increased throughout the study period (APC 11.1; 95% CI: 7.2%-15.2%) and GW presented with increasing trends for the rest of age groups until age 60. Among men aged 20 to 22 years, the increasing incidence of GW shifted to a downward trend in 2013 (APC 2009-2013: 17.0; 95% CI: 8.2%-26.5%; and APC 2013-2016: -4.5%; 95% CI: -14.6%-6.9%). A similar pattern was observed among men aged 23 to 25 years, in which the incidence shifted to a decline in 2014 (APC 2009-2014: 16.0; 95% CI: 12.0%-20.2%; and APC 2014-2016: -6.0%; 95% CI: -18.4%-8.3%). As opposed to GW, in men aged 20 to 25 years the incidence of genital herpes increased significantly over the study period.

References

In our study population, GW have substantially decreased among vaccinated cohorts and there is a shift to a downward trend in young men. A high coverage of a single-cohort HPV vaccination strategy has provided extended benefits through a herd effect of HPV vaccination in non-vaccinated men.

FC 19. Diagnostics & management 2

00427

A NOVEL PATCH SAMPLING APPROACH FOR GRADING & LOCATING CERVICAL LESIONS

19. New technologies

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Background / Objectives

Screening for cervical cancer and its precursors can be facilitated by the detection of HPV DNA. Multiple studies have demonstrated the higher sensitivity of this approach to HSIL. However primary HPV screening has low specificity on its own thus leading to an increase in the number of women that are referred to colposcopy with LSIL. This stems from the fact that mere presence of DNA doesn't correspond to malignant disease. Furthermore, an increase in referrals to a subjective procedure like colposcopy may lead to unnecessary treatment, increasing the risk of neonatal complications due to prematurity.

Results

We utilise a novel patch sampling approach to obtain the surface cells of the cervix (including the transformation zone), while preserving their spatial positions. Patients attending colposcopy had a pre and post-acetic acid application photo, interspersed by patch sampling. 25 patients with a high-grade smear (at time of sampling) and then histology proven HSIL were recruited in one arm vs. 25 patients with LSIL. This patch is then probed with antibodies to the E4 protein (LSIL marker) and p16/MCM (HSIL). The signal for each antibody is then analysed using a mathematical model/ image analysis approach to identify these regions in sheets of cells collected on the patch.

Conclusion

Our novel approach safely samples the cells at the surface of the cervix and can be probed with biomarkers. Our analysis approach is able to identify clusters of cells that are p16/MCM positive, indicating the presence of underlying HSIL, while E4 clusters indicated the presence of underlying LSIL. We were then able to successfully quantify the signal which, correlated with the underlying histological diagnosis. This approach has also been successfully automated thus reducing any operator related

bias that affects current methods. Our approach is also less invasive (compared to cytology) making it more acceptable to women and thus may lead to improved uptake. Finally turnaround time for our approach is only 36 hours, which would enable focusing of resources on women that truly require treatment.

References

Our novel approach of applying biomarkers with preservation of spatial information to localise HPV mediated cervical disease has proven effective in identifying HSIL. Furthermore, the use of this in combination with E4 enables us to quantitatively discriminate HSIL vs. LSIL. Thus, in the context of primary HPV screening this approach has the potential to prevent unnecessary referrals to secondary care. Our non-invasive patch sampling approach has the added potential to be used to monitor patients over time, especially those with CIN2 diagnosis', without the need for multiple, invasive procedures. Finally, the ability to localise lesions has the potential to lead to more personalised treatments.

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00601

Assisted Digital Cervicography (ADC): A New Tool for Clinical Screening of the Cervix

19. New technologies

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Background / Objectives

Methods of cervical cancer screening differ, but include screening through cytology (PAP), Human Papillomavirus (HPV) testing, naked-eye visualization with acetic acid (VIA) or visualization with lugols iodine (VILI), or a combination of these methods. However, no one method of screening is enough to definitively eliminate risk for the development of CIN. 40 studies compared HPV to cytology on over 140,000 women between 20 to 70 years old who attended routine cervical screening. For every 1,000 women screened, there will be 980 women who will not have precancerous changes.[i] However, among the 980 normal women, there are many infections and/or abnormalities that can be found on the cervix visually that may increase the risk of CIN development if the infection remains untreated through increased risk for HPV entry. These infections may not be found on a cytology or HPV test. Identifying these infections is important because a transient period of HPV may begin to replicate in immature metaplasia and/or other infections that can specifically be seen through a visual exam. Viral replication may induce the host cells to proliferate abnormally, potentially leading to CIN.

Results

A digital colposcope was used at the primary clinical screening, alongside cytology and HPV tests to investigate the number of such infections that exist among a normal screening population.

Conclusion

Among the first 100 patients screened with assisted digital cervicography (ADC) during a routine, primary clinical exam in Tel Aviv findings show 15 cases of normal cervixes, 31 cases of normal metaplasia, 24 cases of immature metaplasia, 26 cases of miscellaneous infections, and 4 cases of potential CIN.

References

A new clinical approach called ADC can be adopted, where a digital colposcope is used at the primary clinical screening, alongside cytology and HPV test, where the clinician uses the colposcopic lightsource and magnification, alongside acetic acid and lugol's iodine to visually identify these types of viral infections that can lead to viral replication, and entrance of HPV. The technique is affordable and easy to conduct by general gynecologists who do not need to be colposcopy experts after completing a short, online course. Integrated into the digital colposcope is a system to consult with an expert or tutor so that the patient does not need to have a referral or travel far for routine exams for non-invasive findings. This is a tool to learn the physiopathology of the cervix and to treat benign lesions in order to prevent HPV entry. This also leads to a decrease in stress and anxiety among patients that are simply undergoing routine examination and treatment. This is a new era of clinical screening to prevent CIN development and improve patient care.

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00006

EVIDENCE FOR CLINICAL UTILITY OF EXTENDED HPV GENOTYPING IN PERSISTENCE TRACKING AND FOLLOW-UP AFTER ABNORMAL RESULTS AND COLPOSCOPY AND TEST-OF-CURE

20. Diagnostic procedures / management

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Background / Objectives

Guideline originators have not yet included an analysis of the body of science published recently about the clinical value of extended HPV genotyping (xGT) in persistence tracking, follow-up of women with abnormal results, and follow-up after treatment of high-grade cervical intraepithelial neoplasia (CIN).

Results

PubMed, Cochrane Database of Systematic Reviews, and Health Technology Assessment database were searched from 2001 through 2018 for relevant studies. Hand-searching of retrieved article reference lists supplemented the search. Eligible studies included prospective studies of women and retrospective studies of residual specimens from women that were tested using HPV genotyping tests following an abnormal screening result, or colposcopy, or treatment for high-grade cervical intraepithelial neoplasia. The reference standards were CIN2 or CIN3 or CIN2+ or CIN3+ or invasive cervical cancer. The timeframe for follow-up studies was at least 6-months to determine persistence; periods of 12-months and 24-months were accepted. This systematic review has been registered with PROSPERO. Cochrane risk of bias assessment was performed. GRADE methodology was used to establish quality and strength of evidence.

Conclusion

A PRISMA flow diagram is presented for this systematic review. 31 original research articles met inclusion and exclusion criteria. Reporting xGT results provides profound discrimination of both current and future CIN3+ risks, due to the differential risks of same genotype persistence versus new genotype infection. Within subjects with persistent same genotype, xGT can discriminate risk by more than ten-fold. xGT

could be utilized as follow-up type-specific persistence versus clearance, to support risk-based clinical decisions. Similar management for similar risk-discrimination is benchmarked.

References

Based on quality-evaluated studies that met inclusion criteria, 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. The role of same genotype persistence is critical to test of cure assessments. 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.

References

Note: the author/presenter is a former associate professor of Ob/Gyn, Editor-in-Chief of a peer-reviewed journal; Cochrane reviewer; Senior Scientist in an Evidence-based Practice Center; and current member of GRADE

00028

Sexual Function of Women is not impared by HPV related lesions

20. Diagnostic procedures / management

S. Fornage

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Background / Objectives

While the stressful psychological impact of lesions associated to Human Papillomavirus (HPV) and their diagnosis in women is well known, evidences of their sexual impact are lacking. Our aim is to evaluate the impact of HPV related lesions on the female sexual function with validated questionnaires. Our first outcome was to determine if there was a differences in the FSFI global score and sub-scores between the two populations. Our second outcome was to determine the chararcteristics of female sexual dysfunctions in women with HPV-related lesions.

Results

This is a prospective study comparing the sexual function of women diagnosed for the first time with an abnormal cervical PAP smear or vulvar condyloma (case group) to the sexual function of women with a normal cervical PAP smear (control group) . We used 2 validated questionnaires, the Female Sexual Function Index (FSFI) and the Hospital Anxiety Depression Scale (HADS) mailed to the patients. We excluded women younger than 21 as well as menopausal women and pregnant women.

Conclusion

Forty-eight patients in both groups returned the questionnaires. Populations significantly differ in age, smoking habit, parity and gestity, use of hormonal contraception and vaccination against HPV. Mean FSFI total score is similar in the case group (29.1) and in the control group (28.8) (p=0.3). FSFI scores is <26.55 in 44.4% of the patients of the case group and in 55.6% of the patient of the control group. These differences are not statistically significant. Amongst the FSFI items, arousal score is significantly better in the case group. The others subscores are similar. HADS scores are also similar between the 2 groups.

References

Unlike our clinical experience, global female sexual function is not impared by HPV related lesions. This call into question the choice of the questionnaires and more globally the quantitative approach for evaluation of the female sexual function. Arousal is better in the case group and this difference has to be further studied. We propose the add qualitative studies to further explore the female sexual function in patients with HPV related lesions.

00217

Risk factors for positive margins in transformation zone excision specimens

20. Diagnostic procedures / management

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Background / Objectives

Approximately 10-30% of high-grade squamous intraepithelial lesions (HSIL) of the uterine cervix progress to invasive disease. Transformation zone excision (TZE) effectively reduces the risk of progression. Incomplete excision is associated with higher rates of residual and recurrent lesions.

The aim of this work is to identify risk factors associated with positive margins (HSIL) in TZE specimens.

Results

Retrospective review of the records of patients submitted to TZE, and who had a diagnosis of HSIL in the surgical specimen (2011 to 2018).

Conclusion

Out of 201 patients, 28 had positive margins (13.9%). The endocervix was involved in 60.7% (17/28) of these, while the exocervix was in 21.4% (6/28), and both endo/exocervix in 17.9% (5/28).

Women with positive margins were older (mean age 44.5±14.8 vs. 38.9 ± 9.4 years, $p<0.05$) and were more likely to be postmenopausal [29.03% (9/31) vs. 11.46% (18/157), $p<0.01$].

No differences were found concerning immunosuppression, smoking, previous vaccination against human papillomavirus (HPV), or type of contraception.

Non full visualization of the squamocolumnar junction (SCJ) at colposcopy was associated with positive margins [30.95% (13/42) vs. 8.40% (10/119), $p< 0.01$). If the transformation zone (TZ) was completely endocervical, regardless of visualization or

not of the SCJ, there was also a trend to have positive margins [28.57% (6/21) vs. 13.46% (7/52) if only partially endocervical and 8.86% (7/79) if totally in the exocervix, p=0.059].

Margin status was not associated with the TZ dimensions, location of the lesions, or number of quadrants affected.

No differences were observed when comparing the technique used to perform the TZE (loop vs. needle electrocautery excision) nor with the volume of the specimen.

Higher grade cytologic abnormalities were more frequently associated with positive margins: ASC-H/HSIL 17.29% (23/133) vs. LSIL 2.85% (1/35), p<0,05. On the other hand, HPV genotype was not an independent predictor of margins status.

As the severity of lesions increased, so did the rate of positive margins: CIN2 11.01% (4/73), CIN3/CIS 15.52% (18/116) and invasive carcinoma 66.77% (6/9), p<0.01.

References

Older age, menopausal status, incomplete visualization of the SCJ, ASC-H/HSIL Pap smear, and more severe histology were associated with positive margins in TZE specimens.

No differences were found concerning the dimensions of the specimens obtained.

Contrarily to what has been reported by others, HPV16 was not associated with a higher incidence of positive margins.

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00293

HUMAN PAPILLOMAVIRUS AND MEDICALLY ASSISTED REPRODUCTION: A MULTICENTER PROSPECTIVE STUDY

20. Diagnostic procedures / management

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Background / Objectives

Despite Human Papillomavirus (HPV) is one of the main causal agents of sexually-transmitted diseases, no recommendations exist on the risk related to HPV during Assisted Reproductive Technology (ART) procedures. The main objective of this prospective multicentric cohort study was to evaluate the prevalence of HPV at the different steps of ART program. Secondary objectives were to investigate (1) the efficiency of sperm pellet preparation procedures to eliminate HPV, (2) the correlation between HPV detection in semen and male infertility, and (3) the correlation between HPV detection in males and/or females and success of life birth rate.

Results

A total of 914 consenting couples enrolled in a ART program in Saint-Etienne and Bordeaux University Hospitals was included in the study between 2012 and 2016. Genital HPV screening was performed in males and females using a real-time PCR protocol derived from the INNO-LiPA® HPV Genotyping Extra II assay (Fujirebio) [1]. HPV DNA testing was carried out at the different steps of ART for couples detected positive for HPV, and for newborns in case of pregnancy.

Conclusion

The prevalence of HPV DNA was 17.1% in semen (15.8% in seminal plasma only, 0.7% in the final sperm fraction only and 0.6% in both) and 16.1% in cervicovaginal samples (CVS). The percentages of high-risk genotypes (mainly 31, 39, 51 and 52) were 63.7% and 65% in semen and CVS, respectively. Among HPV positive women enrolled in In Vitro Fertilization program, 26.5% of oocytes punctures and 15.2% of embryos culture media also tested positive for HPV DNA. Four newborns from a total of 253 newborns tested positive for HPV at the throat level, 2 exhibiting similar genotypes as the mother, 1 as the father and the last from parents both negative for HPV. No correlation was found between HPV detection and fertility parameters (in either men or women) or procreation success.

References

Our exhaustive study indicates the presence of HPV DNA of oncogenic genotypes at several steps of ART procedures until child birth. Our findings raise the question of the relevance of a specific management of the risk linked to HPV during ART and of a revision of guidelines in Reproductive Biology Units (in particular the sperm pellet preparation). More broadly, our data may plead in favor of an expansion of HPV vaccination towards males.

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00349

ACCURACY OF COLPOSCOPY AND p16/Ki67 IN THE DETECTION OF HIGH GRADE LESIONS IN HPV-POSITIVE WOMEN

20. Diagnostic procedures / management

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Background / Objectives

The objective of this investigation was to evaluate the accuracy of colposcopy and p16/Ki67 in the detection of high grade disease (HGD), considered CIN 2+, in women with high risk HPV (hrHPV).

Results

Retrospective study of 220 women with positive hrHPV who underwent cervical biopsy in our department, between December/2014 and December/2017. All patients underwent colposcopy.

Conclusion

In our study the mean age was 39,3±10,5 [16-79] years. Cytology results were 20 (9,1%) NILM, 39 (17,7%) ASC-US, 12 (5,5%) ASC-H, 89 (40,5%) L-SIL, 35 (15,9%) H-SIL, 17 (7,7%) AGC and 8 (3,6%) suspicious of carcinoma; 113 (51,3%) were HPV 16 or 18 positive and p16/Ki67 was positive in 138 (62,7%). Colposcopy revealed major abnormal findings in 60,0%. Histological diagnosis after biopsy were: normal in 16 cases (7,2%), 93 (42,3%) CIN1, 68 (30,9%) CIN2, 27 (12,3%) CIN-3 and 9 (4,1%) carcinomas (5 [2,3%] adenocarcinomas and 4 [1,8%] SCC).

Within the group of positive p16/Ki67 there were 85 cases of biopsy-confirmed CIN2+, thereof 30 CIN3+ cases; and only 13 cases of CIN2+ (4 CIN3 cases) within the negative ones. p16/Ki67 showed high sensitivity (86,7%) and modest specificity (55%) in identifying the presence of HGD (CIN2+) at surgical biopsy. Positive and negative predictive values (PPV/NPV) for HGD were 63,4% and 82,2%, respectively

(ROC 0,710). In contrast colposcopy showed 74,8% sensitivity, 52,7% specificity, 59,2% PPV and 69,4% NPV (ROC 0,655). If we consider CIN 3+ lesions the performance of p16/Ki67 and colposcopy was respectively: 91,2% vs. 77,8% sensitivity, 39,7% vs.42,4% specificity, 22,0% vs.20,9% VPP and 95,9% vs. 90,6% NPV.

Making a separate analysis of the performance of p16/Ki67 for CIN2+ between the ASC-US and LSIL we found 86,8% sensitivity, 57,1% specificity and 90,6% NPV (83,3% sensitivity, 76,0% specificity and 90,4% NPV for ASC-US alone and 88,6% sensitivity, 49,2% specificity and 90,6% NPV for LSIL alone). The corresponding results for colposcopy were 77,5% sensitivity, 56,5% specificity and 84,2% NPV for both ASC-US and LSIL (76,9% sensitivity, 57,1% specificity and 80,0% NPV for ASC-US alone and 77,8% sensitivity, 53,3% specificity and 84,2% NPV for LSIL alone).

References

In conclusion double staining showed better performance than colposcopy in predicting HGD, being even better if we consider CIN 3+ lesions. This difference is larger if we consider low grade citologies. As a result it could be a usefull tool as an adjunct to cytology in the triage of hrHPV eventually decreasing the need for colposcopy referrals.

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00384

THE ADDED VALUE OF RESCREENING CYTOLOGY NORMAL SAMPLES WITH POSITIVE HPV MRNA TEST FOR THE DETECTION OF CIN2+ IN PRIMARY SCREENING

20. Diagnostic procedures / management

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Background / Objectives

Objectives. To estimate the increased detection rate of CIN2+ in women with normal Pap-smears by rescreening Pap-smears from HPV mRNA positive samples.

Results

Methods. From April 4th, 2013, the Department of Pathology, Ålesund Hospital, introduced a study by rescreening all normal Pap-smears that had a positive HPV mRNA test (NorChip PreTect SEE) (types 16, 18 and 45) in women younger than 40 years. Within the SymPathy database, a study population of 4 366 women aged 23–39 years with no prior history of CIN1+ was established.

Conclusion

Results. 38% of women with normal cytology were tested via HPV mRNA (1444/3851), and 28 samples were positive (1.9%). After re-evaluation of the index cytology and subsequent follow-up smears, 15 women had colposcopy, resulting in five diagnoses of normal biopsies, 6 CIN1 and 9 CIN2+. The detection rate of CIN2+ among rescreened normal Pap-smears was 0.62% (95% CI: 0.60–0.65). In the ASC-US+ arm (n=515), 138 CIN2+ were detected. If we apply the CIN2+ detection rate among cytology normal / HPV mRNA-positive women (0.62%) to the arm of women with normal cytology without HPV testing, a 17-18% increase in CIN2+ detection rate was estimated. Four cancers were detected in the ASCUS+-arm, none among rescreened SEE-positives.

References

Conclusions. By testing all women with normal cytology with a specific HPV mRNA test, a significant increase in screening program sensitivity can be achieved. The volume of rescreened smears (1.9%) is very low. In addition, the study adds quality to educating the screeners by rescreening presumably false negative Pap-smears.

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00534

LOW PROPORTION OF UNREPORTED CERVICAL TREATMENTS IN THE CANCER REGISTRY OF NORWAY

20. Diagnostic procedures / management

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Background / Objectives

Accurate information about treatment is needed to evaluate cervical cancer prevention efforts. We evaluated reasons of being without recorded cervical treatment in the Cancer Registry of Norway (CRN).

Results

We identified 47 423 (92%) high-grade cervical dysplasia patients with treatment and 3 983 (8%) without treatment in the CRN in 1998–2013. We linked the latter group to the nationwide registry of hospital discharges in 1998–2015. Of patients still without treatment, we selected randomly 375 for review of their medical history. Factors predicting incomplete treatment records were assessed by multiple imputation and logistic regression.

Conclusion

Registry linkage revealed that 10% (401/3 983) of patients received treatment, usually conisation, within one year of their initial high-grade dysplasia diagnosis. 11% (n=44) of those were missing due to unreporting and 89% (n=357) due to misclassification at the CRN. Of all cases in medical review, patients under active surveillance contributed almost 60% (223/375). Other reasons for unregistered treatment were uncertain dysplasia diagnosis, invasive cancer or death. Coding error occurred in 19% (73/375) of randomly selected cases. CRN undercounted receipt of treatment by 38% (n=1 526) among patients without registered treatment which translates into 97% overall completeness of treatment data. Incomplete treatment records were particularly associated with public laboratories, patients aged 40–54 years, and the latest study years. The average annual number of conisations recorded in the CRN has increased from 3,900 in years 2010-2013 to 5,700 in years

2014-2016. This might reflect a decrease in incomplete recording of treatment data and/or true increase in number of conisations nationwide.

References

CRN records close to complete treatment data on cervical treatments, with missingness largely being due to misclassification. Validity of treatment data has been identified as a high-priority task in registry linkage

00188

Can Thin HSIL of the Cervix Progress to Invasion?

22. Cervical neoplasia

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Background / Objectives

Thin high-grade squamous intraepithelial lesions (HSIL) of the cervix are a variant of HSIL that are <9 cells thick (1). Thin HSILs develop in early immature metaplastic squamous epithelium of the transformation zone (TZ) without anteceding low-grade squamous intraepithelial lesions (2). The risk of thin HSIL progressing to invasion is unclear.

Results

We studied 34 consecutive conization specimens containing microinvasive (FIGO stage Ia1) squamous cell carcinoma (SCC) of the cervix.

Conclusion

All early invasive foci were located inside the TZ and arose more often from HSIL in endocervical glands than from HSIL at the ectocervix. Early stromal invasion originated from a field of thick HSIL in 32/34 (94%) specimens, only 2 (6%) specimens showed early stromal invasion originating from a field of thin HSIL of 7-9 cells thickness. No early stromal invasion originated from thin HSIL 4-6 cells thick.

References

Our findings indicate that the risk of invasion of thin HSIL is low, and it may be that women with biopsy-confirmed thin HSIL may be candidates for expectant follow-up. We postulate two latency periods between thin HSIL and microinvasive SCC: the first between thin HSIL and thick HSIL. Epithelial thickening occurs due to human papilloma virus E6 and E7 gene-induced clonal expansion with intraepithelial proliferation of neoplastic transformed cells. The second latency period is between thick HSIL and invasive SCC.

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00515

CONSERVATIVE APPROACH IN THE MANAGEMENT OF YOUNG WOMEN WITH CIN2

22. Cervical neoplasia

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Background / Objectives

The better understanding of the natural history of cervical dysplasia is leading to the recommendation of more conservative approaches. Given the significant chance of
regression of CIN2 in young women and, on the other hand, the increased risk of negative obstetrical outcomes associated with its treatment, especially transformation zone excision (TZE), makes conservative approach an appealing approach.
Primary objective: Evaluation of the probability of CIN2 regression in untreated women under 30 years old.
Secondary objectives: Evaluation of the time until regression/progression and potential risk factors related to outcomes.

Results

Retrospective evaluation of the cases of women younger than 30 years old, with a diagnosis of CIN2 and who opted for conservative management (2012-2018). Follow up consisted of Pap smear, HPV test, colposcopy and biopsy according to colposcopy findings, every 6 months.
Regression was considered when there was no histological evidence of CIN2+ at 24 months. χ 2 test was used to evaluate categorical variables an t student for continuous ones; p values <0,05 were considered significant.

Conclusion

Out of 885 patients managed during this period, 15 were eligible for analysis.The mean age at diagnosis was 26.1±1.8 years old (22-29), 9 were smokers, and none was immunosuppressed.
Seven women were nulliparous at the time of diagnosis; 9 were combined oral contraceptive (COC) users; only one had history of previous HPV vaccination.
Regression was achieved in 5 cases; in 5 there was indication for TZE (CIN2

or CIN3); 5 are still in follow up. There were no cases of invasion.
In the 5 cases of regression, in 3 it occurred at 6 months, in one at 12 months, and another at 18 months. In three cases the genotype involved was the HPV16. HPV testing became negative in four cases (one at 6 months, two at 12 months and one at 24 months). Two of the cases in the regression group still have CIN1/LSIL.
There were 3 pregnancies in the group of women that had regression; one before and two after the regression.
Comparing the ones who had regression with the others, there were no differences concerning age at diagnosis, menarche, parity, use of COC, smoking, previous vaccination, referring Pap smear result, or HPV16 involvement.

References

Conservative management can be offered to compliant women, younger than 30 years, after a diagnosis of CIN2.
In this small sample it was possible to avoid TZE in almost half of the cases, with potential obstetric benefits.
The adoption of the LAST terminology may lead to a more aggressive and unnecessary approach in this population.

00001

NEW THERAPEUTIC REMOVAL APPROACH OF HEAVY FORMS OF CONDYLOMA WITH HIV, HEPATITIS AND IMMUNO COMPROMISED PATIENTS, WITH ADDITIONAL PROTECTION FROM PROFESSIONAL HAZARD TO THE DOCTOR PENDING PROCEDURE

30. Sexually transmitted diseases and HIV infection

I. Jeremic

Ordination Jeremic (Serbia)

Background / Objectives

HPV is the epidemic of the modern age, influenced to great extent by easy transmission of the infection.

With imuno compromised patients, and also those undegoing immuno suppresive therapy, (Corticosteroids and chemo therapy), incubation period is decreased to 3 weeks to three months.

It presents a therapuetic challenge due to the following:

1. Sensitivity of genitoanal region to forced trauma
2. Inaccesibility of the area, intraanal –vaginal and cervical condyloma
3. High vascularazitaion of vagina, cervix and hemorrhoids
4. Suceptibility of infection, bacterial flora (vagina and colon).
5. Weak immunity
6. Professional hazard

Results

The study included 100 patients of both gender 16 to 50 years of age, HIV and HbsAg positive, patients under immune suppressive chemo therapy, with medium

and heavy forms of condyloma on all parts of genitoanal region, with emphasis on cervix, vagina, anal and intraanal localization.

New technique of work employs two special types of radio access vaporization.1.Radio wave vaporization which involves the evaporation of cells infected with HPV virus, and Radio wave melting of the Condyloma masses is the second type of evaporation, it is used to moderate the medium and heavy forms of genital wart infections.

Conclusion

Therapeutic results of the new method are:

1. Almost bloodless operating field

2. Total precision and control in removing of all forms of genital warts in one act

3. Lateral damage to healthy tissue is less than 10 microns-

4. The recurrence rate is less than 3%

5. All interventions are performed in local anesthesia-duration up to 10 min

6.The therapy of choice for the pregnant women and immune deficient people

7.The working technique that protects doctors from the occupational exposure from HIV and positive Hepatitis B and C patient

References

With conditions of general immunodeficiency, with HPV infection in progress, the employment of new radio wave technique of condyloma removal ensured the principal precondition of successful therapy which consists of preservation of local immunity in the treated genitoanal region.

Since these patients have a problem with boosting of the immunity in general , new technique provides us with a solution and treatment of HPV infection in one go without complications such as infection, bleeding, pains, and heavy relapse.

References

In 2010- I was appointed Licensed Educator of radio wave surgery for Europe - Turkey and Russia in the field of gynecology and dermatosurgery by an expert team of doctors in New York.

I joined this team in 2012

Patented a special technique of radio access LOOP excision in young girls who have not given birth with severe precarcinoma on the cervix (CIN II, III) without narrowing and shortening of the cervical canal

4. Patented a special radio wave technique removing heavy form of Condyloma in both sexes at vagina mucosa, labia, anal and intraanal involving special bloodless of melting Condyloma mass with lateral damage to healthy tissue below 10 microns

Case studies are also presented at Medical Faculties in the USA as examples of treatment of first choice

The founder and the owner of "Polyclinic Jeremić" the Educational Center of radio wave surgery for Europe

FC 20. Low income countries

00071

HIGH PREVALENCE OF HUMAN PAPILLOMAVIRUS, HIV AND OTHER STI AMONG MEN WHO HAVE SEX WITH MEN IN TOGO IN 2017

02. Epidemiology and natural history

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Background / Objectives

No data are available on HPV prevalence in Togo. The aim of this study was to assess HPV, HIV and other STIs prevalence in Togo among the key population of men who have sex with men (MSM).

Results

A cross-sectional study was conducted in 2017 among MSM recruited in 4 cities of Togo (Lomé, Kpamilé, Atakpané and Tsévié) through the respondent driven sampling method. Participants' socio-demographic characteristics and sexual behaviors were recorded using a standardized questionnaire. Anal swabs were collected and sent to France to test HPV (Anyplex II^{TM}HPV28 Detection test detecting 19 high risk (hrHPV) and 9 low risk (lrHPV) HPV) and 7 STIs (Allplex^{TM}STI Essential Assay). HSV-1/2 were detected using RealStar®alphaHerpesvirus PCR Kit. HIV, Syphilis and HBs Ag screening were performed in Togo with SD Bioline HIV/Syphilis Duo® and Alere Determine^{TM}HBsAg tests.

Conclusion

207 MSM with a median age of 22 years (IQR=20-25) were enrolled. Prevalence of each STI is shown in the table.

	HIV	any type HPV	hrHPV	*N.gonorrhoeae* (NG)	*C.trachomatis* (CT)	*M.genitalium* (MG)	HSV-2	HBV	Syphilis
Prevalence (n=207)	26.1%	52.7%	44.9%	11.6%	9.7%	15.0%	8.7%	3.4%	0%

The any type and hrHPV prevalence were significantly higher among HIV-positive MSM compared with HIV-negative MSM (any type HPV: 88.9% vs 31.9%, $p<10^{-5}$; hrHPV: 85.2% vs 30.7%, $p<10^{-5}$). Other STIs, except HBV, were also more prevalent among HIV-positive MSM group (NG, p=0.03; MG, p=0.04; HSV-2, p=0.001 and a trend for CT, p=0.06). Having at least one hrHPV type detected in the anal canal was significantly associated ($p<10^{-5}$ for all) with the detection of other STIs, except HBV. Prevalence of each STI was significantly higher in MSM from Lomé than those recruited in other cities, excepted for HBV and HSV-2 (HIV, p=0.002; hrHPV, $p=2.10^{-5}$; NG, p=0.01; MG, p=0.04; CT, p=0.005). Most common hrHPV types were HPV35 (15.0%), HPV16 (13.0%), HPV31 (12.6%) and HPV59 (11.1%). Most common lrHPV were HPV6 (25.6%), HPV42 (17.9%), HPV40 (7.3%) and HPV11 (5.3%). HIV-positive MSM were more likely to have multiple any type HPV infections than HIV-negative MSM (85.2% vs 28.7%, $p<10^{-5}$). In multivariate analysis, HIV infection (aOR: 10.1, 95%CI: 4.0-25.6), HSV-2 anal excretion (aOR: 26.7, 95%CI: 2.9-244.3), CT anal infection (aOR: 11.7, 95%CI: 2.3-58.9), MG anal infection (aOR: 9.6, 95% CI: 3.1-29.9) and living in Lomé (aOR: 2.8, 95%CI: 1.1-7.1) were associated with increased risk of anal hrHPV infection. All participants with anal NG infection (n=24) were infected with at least one hrHPV.

References

These first data on HPV in Togo report high prevalence among MSM, highlighting the critical need of implementation of a national strategy regarding vaccination against Papillomavirus. We also described an unusual distribution of HPV types, with HPV35 being the most prevalent hrHPV.

00072

CERVICAL AND ANAL HUMAN PAPILLOMAVIRUS, HIV AND OTHER STI PREVALENCE AMONG FEMALE SEX WORKERS IN TOGO IN 2017

02. Epidemiology and natural history

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Background / Objectives

In West Africa, limited data are available on HPV prevalence and on concomitant anal and cervical HPV infections. The aim of this study was to assess the prevalence of HPV, HIV and other STIs among female sex workers (FSW) in Togo.

Results

A cross-sectional study was conducted in 2017 among FSW recruited in hot spots (bars, clubs, streets, brothels) in 4 cities (Lomé, Kpamilé, Atakpané and Tsévié) following the respondent driven sampling method. Cervical and anal swabs were collected and sent to France to test HPV (Anyplex II^{TM}HPV28 Detection detecting 19 high risk [hrHPV] and 9 low risk HPV [lrHPV]) and 7 STIs (Allplex^{TM}STI Essential Assay). HIV and Syphilis antibodies screening were performed in Togo with SD Bioline HIV/Syphilis Duo^{TM}.

Conclusion

310 FSW with a median age of 25 years (IQR=21-32) were enrolled. Median ages of first sexual intercourse and of sex work initiation were 17 years (IQR=15-23) and 19 years (IQR=17-23), respectively. Prevalence of each STI is presented in the table.

	HIV	HPV any type	hrHPV	*N.gonorrhoeae* (NG)	*C.trachomatis* (CT)	*M.genitalium* (MG)	*T.vaginalis* (TV)	Syphilis
Prevalence (n=310)	10.6 %	45.2 %	39.7%	4.2%	6.1%	5.5%	6.5%	0.6%

Any type HPV prevalence was significantly higher among HIV-positive than HIV-negative FSW (63.6% vs 43.0%, p=0.03). Prevalence of other STIs were significantly higher in hrHPV-positive FSW for NG (p=0.004), MG (p=0.01) and showed a trend for TV (p=0.06) and CT (p=0.1). Prevalence of hrHPV, MG and TV were higher in FSW enrolled in Lomé than in other cities (p=0.02, p=0.04 and p=0.004 respectively). The most prevalent hrHPV types were HPV53 (6.5%), HPV58 (6.1%), HPV35 (5.8%), HPV31 (5.5%) and HPV16 (4.8%). The most prevalent lrHPV was HPV42 (6.5%). HPV6 and HPV11 prevalence were 3.2% and 0.6% respectively. Multiple hrHPV infections were more frequent in HIV-positive than in HIV-negative FSW (30.3% vs 14.8%; p=0.04). Both anal and cervical swabs were collected for 276 FSW. Prevalence of hrHPV was significantly higher in cervical than in anal swabs (40.2% vs 27.9%, p=0.003). hrHPV anal infections were significantly more frequent in HIV-positive than HIV-negative FSW (63.0% vs 24.1%, p<0.001). Prevalence of cervical hrHPV decreased with FSW's age while it was relatively stable for anal hrHPV prevalence. Concomitant anal and cervical hrHPV infections were present in 37% of hrHPV positive FSW but concordance was low (kappa coefficient=0.3).

References

These data report an unusual distribution of hrHPV types, with HPV16 only at the fifth rank. This study also reports one of the first analysis of both cervical and anal samples showing a high rate of concomitant HPV infections with low concordance. These findings highlight the critical need of implementation of a national strategy regarding vaccination against HPV in the female population.

00161

PREVALENCE OF HUMAN PAPILLOMAVIRUS AND OTHER SEXUAL TRANSMITTED INFECTION IN WOMEN FROM LAKE TURKANA AREA, KENYA

02. Epidemiology and natural history

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Background / Objectives

Invasive cervical cancer (ICC) is the most common women malignancy in Kenya. Scarce information is available about the high risk-HPV genotypes attributed to cervical cancer and sexual transmitted infections in the Lake Turkana area.

The objective of the study was to assess the prevalence and distribution of HPV types, Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis in cervical smears from women in north of Kenya

Results

A total of 161 women aged 13–75 years, visiting the Lodwar District Hospital, Kenia without gynecological medical purpose, were invited to participate in the study. All participants completed a detailed questionnaire and underwent a physical examination. Cervical specimens were tested for hr-HPV detection and genotyping by Multiplex Fluorescent-PCR F-HPV typing (Molgentix) and STI Allplex (Seegen) test for Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Trichomonas vaginalis (TV).

Conclusion

The overall HPV DNA prevalence was 31.2%. The most frequent type was HPV58 detected in 38% of positive cases (95% CI: 25.4–51.9), followed by HPV52 detected

in 12% (95% CI: 9.1–30.4), HPV16 and HPV-35 detected in 12% (95% CI: 5–23.3), HPV18 detected in 8% (95% CI: 2.5–18.1) and HPV33 detected in 8% (95% CI: 2.5–18.1).

The overall prevalence for STIs (CT, NG and TV) was 4.3% (95% CI: 4.1–11.2), 3.12% (95% CI: 3.8–8.8) for CT and 1.25% (95% CI: 3.8–8.8) for TV. NG was not detected. Among hr-HPV positive women, 6% (95% CI: 13.1–26.3) had STIs, while among HPV negative women was 3.6% (95% CI: 3.3–11.7). Six women declared to be HIV positive.

References

The high STIs prevalence observed in this population, suggests the need for health interventions to reduce the risk of infections.

00491

HPV VIRAL TEST IN PRIMARY SCREENING OF UTERUS CERVICAL CANCER AT THE NABIL CHOUCAIR HEALTH CENTER IN SENEGALESE WOMEN BETWEEN 30 AND 65 YEARS OLD.

09. HPV screening

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Background / Objectives

To describe the socio-demographic aspects of clients who have been screened for cervical cancer by an HPV viral test; Specify the diagnostic, therapeutic and follow-up aspects of the clients who had a positive test.

Results

This was a prospective, descriptive and analytical study conducted at the Nabil Choucair health center and at Ouakam Military Hospital from 01 May 2017 to 30 January 2018.

The study involved 144 patients who had received an HPV ABBOOT m2000 viral test.

The parameters studied included sociodemographic characteristics, clinical aspects, test results, diagnostic and therapeutic aspects, and follow-up.

The data was collected on a form and the statistical analysis was carried out by Epi-info 7.

Conclusion

We collected 144 women. The mean age of the patients was 39.9 years with extremes of 30 to 55 years. Patients were predominantly married (84%) and housewives predominated (48.1%) and slightly more than half (55.6%) were in school. More than half of the patients 61.8% were on contraception. Almost all patients (92.4%) were in genital activity. The average gestational age was 3.4 with and an average parity of 2.7. In our series, 103 patients (50.7%) had a history of abortions. The average age at marriage was 22.6 years and monogamous households predominated (56.8%). The average age at first intercourse was 22.1 years. The average age at first pregnancy was 23.9 years. More than ¾ of women (78.3%) had a partner; however, note that 21.7% of patients had 2 or more partners. The viral test was positive in 17 patients (11.8%). Papillomaviruses 16 and 18 were the most recovered. Colposcopy was normal and satisfactory in 9 patients (53%), 2 patients had atypical transformation of grade and 2 cases grade 2 (11.7%) 3 (17.6%) patients had viral colitis. Cervical biopsy was performed in 2 (11.7%) patients and histology showed CIN3 and microinvasive carcinoma. Therapeutic 02 conizations were performed. The postoperative course was simple.

References

The HPV viral test in primary screening for cervical cancer offers opportunities and is possible in developing countries such as Senegal despite limited means.

Keywords: HPV viral test, Nabil Choucair Health Center, Cervical cancer screening

00380

RESULTS OF A CERVICAL CANCER SCREENING PILOT STUDY IN MOROCCO COMPARING HPV ONCOPROTEIN E6 EXPRESSION TESTING AND VIA.

13. Screening methods

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Background / Objectives

MorocOncoE6 is a pilot study by the “Centre Mohammed VI pour le Traitement des Cancers”, Casablanca, Morocco and the “Clinic for Gynecology and Gynecological Oncology”, Charité-Universitätsmedizin Berlin, Germany, with support by the Ministry of Health, and Lalla Salma Foundation, Morocco. The primary objective of this study was to test feasibility of OncoE6™ Cervical Test (“OncoE6”; Arbor Vita, Fremont, CA, USA), including patient´s acceptance, compliance, and quality of the test results in comparison to visual inspection with acetic acid (VIA).

Results

This prospective cross-sectional study recruited women from 8 health posts (4 rural, 4 urban) in the Tangier area. All subjects were tested by OncoE6 and by VIA; for OncoE6, a physician and a self collected specimen were obtained from all subjects. All participants underwent colposcopy, and if indicated a biopsy and histology in a local pathology laboratory. The acceptability of sample self-collection was assessed via a semi-structured questionnaire to evaluate knowledge on cervical cancer, socio-demographic characteristics, medical history, and acceptability of the Evalyn brush-based self-collection. All study data were pseudonymized prior to analysis. The data

of the clinical results were given to the patients and follow-up visits and treatment have been established.

Conclusion

216 women of from rural (100) or urban (116) settings have been recruited. 15.5% (n=31) women had a positive/suspicious result on VIA, and 2.3% women tested positive on OncoE6 (self-collected or physician-collected). Multiplex HPV genotyping done at the Charite-Universitätsmedizin (Berlin, Germany) revealed high-risk HPV+ at 11.8% (n=21), with HPV16+ at 5.7% (n=12); other HR-HPV types detected were 51 (n=2), 66 (n=3), 68 (n=1), 73 (=1), 82 (n=1) with three subjects showing multiple infections. 3.1%, were diagnosed at CIN1, while no cases of CIN2 or worse have been observed. 82% of women provided a self-collected specimen, and 68% felt self-collection was straight forward. A control cohort of 20 patients with histologically confirmed invasive cervical cancers recruited at the Cancer Center in Casablanca also underwent OncoE6 testing.

References

This pilot study demonstrated a high degree of acceptability among the participating Moroccan women for cervical cancer screening and for self-sampling. VIA resulted in a substantial number of false positive test outcomes, which could cause costly overtreatment. The existing infrastructure in the target areas may allow for wider implementation of the OncoE6™ Cervical Test. A validation study in a multicenter and multiregional setting is planned.

00517

LOW-COST DIAGNOSTIC FOR THE IDENTIFICATION AND TYPING OF HUMAN PAPILLOMAVIRUS TO SUPPORT CERVICAL CANCER SCREENING IN LOW-RESOURCE SETTINGS

19. New technologies

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Background / Objectives

Human papillomavirus (HPV) infection is responsible for nearly all cervical cancer cases. The lengthy progression from infection to cancer makes screening highly effective in reducing cervical cancer cases and related deaths. Cytology-based screening programs have significantly reduced the burden of cervical cancer in developed regions. However, cytology-based screening must be performed frequently and is poorly suited to low-resource settings, where the vast majority of cervical cancer cases and deaths occur. Molecular HPV diagnostics are gaining usage but they are expensive and use is generally limited to centralized laboratories. For screening programs to achieve the same level of success in low-resource settings, an assay with high sensitivity and predictive value over a long period is imperative. Global Good and QuantuMDx are developing a point-of-care molecular diagnostic for the detection and genotyping of thirteen individual high-risk HPV (HR-HPV) types.

Results

The cassette-based assay is fully integrated, enabling sample to results in under one hour. The cassette runs on a portable, low-cost, battery-operated device. A vaginal or cervical swab is collected from a patient, the swab is placed in liquid medium, and a portion of the sample is loaded onto the cassette. Sample processing, target amplification, and target detection via DNA microarray hybridization occur on cassette without further user interaction. The assay detects a cellular control and genotypes thirteen HR-HPV types if present, which allows for risk stratification.

Conclusion

To date, we have assessed assay performance using contrived and patient samples, including vaginal and cervical swabs from two cohorts. Limit of detection is at or below 100 copies per reaction for each HR-HPV type in patient samples, no cross-reactivity has been detected with low-risk HPV types or non-HPV DNA, and assay performance is comparable with vaginal and cervical samples. Preliminary patient data are concordant with clinically-approved reference methods.

References

We expect the simplicity, affordability, and risk stratification provided by this assay to enable same day diagnosis and management at point-of-care.

00118

Introduction and Evaluation of A Simplest and Fastest Cervical Cancer Screening Technology for Resources Limited Area

35. Low resource settings

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Atila BioSystems, Inc. (United States of America)

Background / Objectives

Early HPV screening and treatment of precancerous lesions is known to be highly effective in drop of cervical cancer death rate. However, HPV infection is still a global issue, especially in poor resources area, even though there are many HPV screening technologies available. The main reason is that sample treatment to extract DNA for HPV detection is very expensive, time consuming and complicated, not to mention complicated expensive equipment required for HPV detection that limits the HPV screening globally. In contrast, AmpFire HPV technology has the simplest sample process (Atila BioSystems, an isothermal amplification to detect all 15 high risk HPV in a single tube reaction and simultaneously genotype HPV 16 and HPV 18, plus an internal control). It doesn't require extracting DNA, simply heats to lyse the samples and then it is ready for detection. In addition, amplification is extremely fast (40 minutes). AmpFire HPV can be finished within an hour from sample to answer (10 minutes heating). The simplicity and speed of AmpFire HPV assay will be a great fit for poor resources area for global HPV screening.

Objective: to evaluate the agreement of HPV detection between AmpFire HPV test and HC2 and their performance for HPV screening.

Results

Methods: A total of 80 patients samples were studied (samples collected in ThinPrep solution by Lehay Clinic, Burlington, MA). The AmpFire assay detects HPV virus by centrifuging 1ml ThinPrep sample solution, discharging the supernantant and then, the cell pellets were heat treated in a Atila lyse buffer for 10 minutes without DNA extraction. 2ul lysed simple was mixed with reaction solution for real time fluorescent detection in an hour. HC2 detection was done by Lehay Clinic following manufacturer's instruction.

Conclusion

Results: Comparing HPV results of Ampfire HPV to HC2, amount the 80 samples, 78 samples are agreed with each other (56 positive samples and 22 negative samples). One sample AmpFire is positive, but HC2 is called negative with cutoff value at 0.62 that is larger than other reported positive cutoff value at 0.2. The other disagreement sample detected by AmpFire as negative, but HC2 report as positive without reporting cutoff value from Lehay Clinic. The %OA (overall agreement) is 97.5%. %PA (positive agreement) was 98.2, %NA (negative agreement) 95.5%.

References

Conclusions: The Ampfire HPV assay performed equally well as HC2. Ampfire HPV assay did not require DNA extraction with very simple sample process and yielded results rapidly within an hour.

00234

PREVALENCE AND RISK FACTORS OF HPV AND OTHER SEXUALLY TRANSMITTED INFECTIONS AMONG 2000 WOMEN IN RURAL GHANA – FINAL RESULTS FROM THE ACCESSING* STUDY

35. Low resource settings

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Background / Objectives

Determine the prevalence and risk factors of 28 mucosal HPV and 18 other sexually transmitted infections among 18-65 year old women living in the North Tongu District, Ghana.

Results

This population-based study included 2000 women, representatively selected by geographical distribution, consenting to self-collect vaginal samples (Evalyn brush) and answer a questionnaire. Extracted DNA was HPV genotyped and presence of STIs determined by multiplex PCR and Luminex bead-based hybridization. Stata was used for regression analysis.

Conclusion

Median age of participants was 32 yrs (range 18-65 yrs). Majority of women had completed Junior High School, worked as traders/farmers, earned less than ~18€ per month, were married and had a median of 2 children. 1943 valid DNA samples were analyzed for HPV and 1937 for STI. High-risk HPV prevalence was 32.3% (95% CI: 30.2-34.5%), 9.7% (95% CI: 8.4-11.1%) of the women had multiple high-risk infections. The 5 most prevalent HPV types in descending order were 16, 52, 35, 59,

and 56. Risk factors by multivariate analysis were young age, not being married, and higher number of sexual partners.

Prevalence of *Chlamydia trachomatis* (CT) was 4.8% (95% CI: 3.9-5.9%), 2.5% (95% CI: 1.8-3.3%) for *Neisseria gonorrhoeae* (NG), and 4.1% (95% CI: 3.3-5.1) for *Trichomonas vaginalis* (TV). *Treponema pallidum* (TP) was detected in one sample only. NG and TV were associated with high-risk HPV infection.

References

Prevalence of high-risk HPV infections are higher than expected from the WHO/ICO HPV Information Centre estimates for West Africa (17.9%). Possible reason is an overrepresentation of women up to 34 years of age, who also happen to have the highest prevalence, compared to the general population in the district. Risk factors identified, such as young age, not being married, and higher number of sexual partners could be confirmed in this study. Condom use could not be assessed, as majority uses non-barrier methods (30%) or no (66%) contraceptive. Prevalence reported for WHO African Region for NG with 2.3% is similar, on the contrary WHO reported a CT prevalence of 2.6%, which is lower than our results. TP with only one case is far below the WHO reported seroprevalence of 3.5%. Differences may also reflect the fact that our sample is not representative of WHO African Region.

The high HPV prevalence highlights the need to increase efforts to vaccinate and screen women, as many are at risk of developing cervical cancer. STI and HPV prevalence seen are representative for the North Tongu District, Ghana and may vary from reported data for Western Africa. Given this context these results can guide future public health policy.

FC 21. Economics & modeling

00266

COST-EFFECTIVENESS OF PRIMARY HPV SCREENING WITH OR WITHOUT DUAL STAIN CYTOLOGY FOR CERVICAL CANCER

32. Economics and modelling

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Background / Objectives

Primary HPV testing could increase the detection rates of cervical intraepithelial neoplasia grade 2+ compared to cytology and it has been recommended as a cervical cancer screening option. The overtreatment due to the high sensitivity of HPV test still has the problem. The concept of dual stain has been introduced to decreased overtreatment case. The objective of this study is to identify the optimal cost-effective strategy for cervical cancer screening program by comparing the different algorithms which based on the use of primary HPV assay.

Results

We use a Microsoft Excel-based spreadsheet to calculate the number of accumulated cases of cervical intraepithelial neoplasia (CIN), invasive cervical cancer (ICC) and the budget impact of each screening program. The model was developed to determine the cost-effective of three screening strategies; pooled HPV test alone, HPV genotyping with reflex dual stain and pooled HPV test with dual stain. All strategies were considered a 5-year interval. Clinical parameters were combined data from the literature to estimate the performance of screening tests and natural history parameters. We assessed direct medical cost including screening and treatment cost. The main outcomes were the total cost, incremental quality-adjusted life years (QALYs) and incremental cost-effectiveness ratios (ICERs).

Conclusion

Strategy entailing HPV genotyping with reflex dual stain is the least costly strategy (total cost $37,893,407) and provides the similar QALY gained compared with pooled HPV alone with reflex dual stain (Average QALY 24.03). The two strategies which using reflex dual stain could increase screening performance and lower the prevalence of cervical cancer than pooled HPV alone. Pooled HPV test with reflex dual stain is more costly compared with pooled HPV test alone without reflex dual

stain. The incremental cost-effective ratio (ICER) was $10.09 per QALY gained. One way sensitivity analysis showed that the model is sensitive to the cost of dual stain and the cost of cancer treatment.

References

Decreasing the incidence of cervical cancer case and increasing the QALYs can be successful using dual stain cytology as the triage test for pooled HPV test or HPV genotyping. The result of our analysis favors the use of HPV genotyping with reflex dual stain as it offers the most QALY at the lowest cost.

00314

COST-EFFECTIVENESS EVALUATION OF HPV SELF-SAMPLING OFFERED TO NON-ATTENDEES IN CERVICAL CANCER SCREENING IN SWITZERLAND

32. Economics and modelling

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Background / Objectives

About 30% of women who are eligible for cervical cancer (CC) screening remain un-screened or under-screened in Switzerland. Human Papillomavirus (HPV) testing on self-collected vaginal samples (Self-HPV) for CC screening has shown to be more sensitive than cytology-based screening and to reach non-attendees. The objective of this study was to explore the cost-effectiveness of a Self-HPV-based screening in Switzerland.

Results

A recursive decision-tree with one-year cycles was used to model the life-long natural HPV history. Markov cohort simulations were used to assess the expected outcomes from the model. Three strategies were compared with the absence of screening: Self-HPV and triage with Pap cytology (Self-HPV/PAP), Self-HPV and triage with colposcopy (Self-HPV/colpo), conventional cytological screening and triage with HPV (PAP/HPV). Sensitivity analyses for the key parameters of the model were conducted to check the robustness of findings. Analyses were performed from the direct health care cost perspective, regulated by the Swiss tariff system.

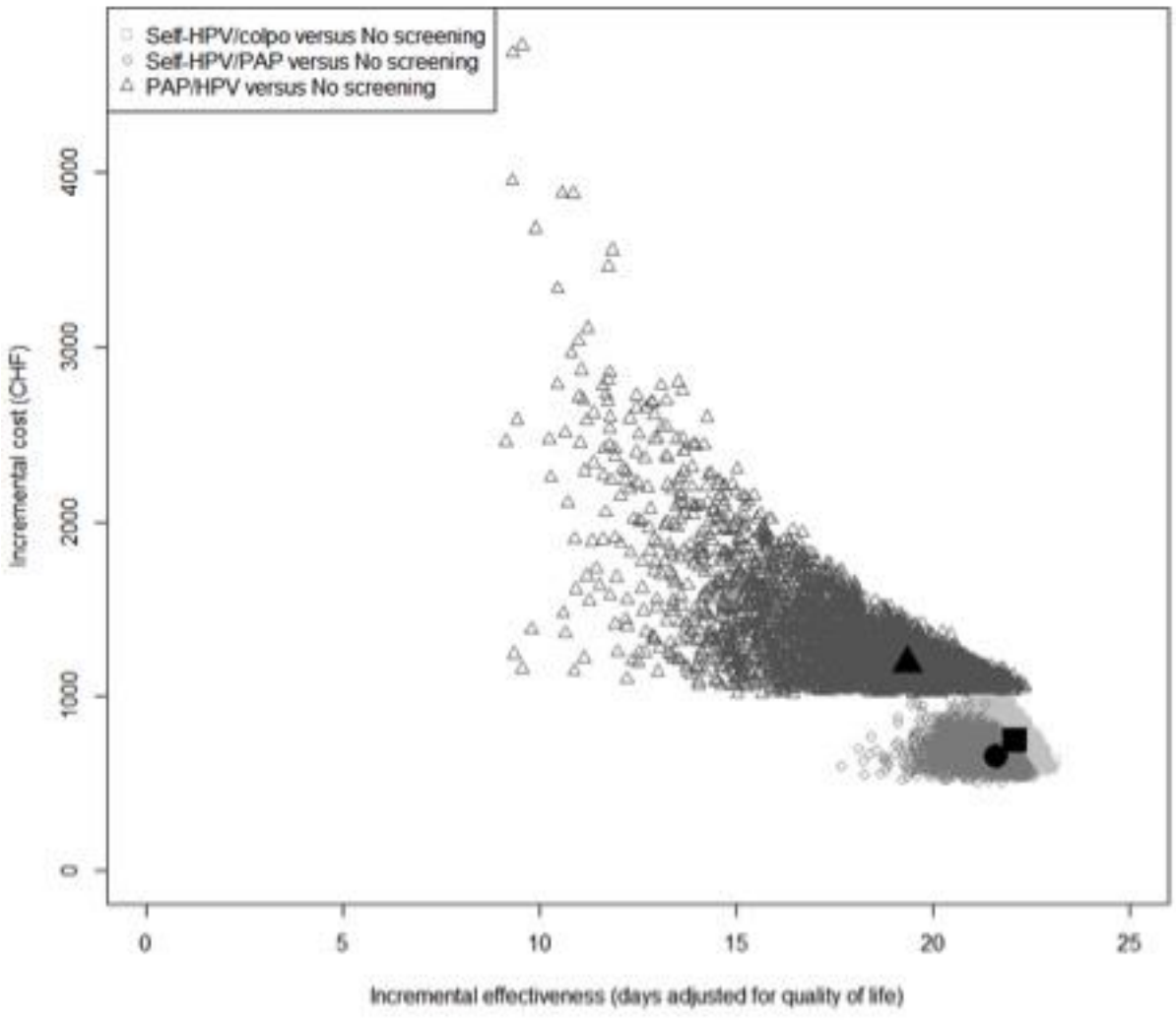
Conclusion

Compared with the absence of screening, offering a Self-HPV screening could prevent over lifetime 86 to 90% of CC and 90 to 91% CC-related deaths. Comparing to the currently cytology-based program, Self-HPV could reduce by 22 to 28% the lifetime CC cases and by 28 to 35% the number of CC-related deaths. Incremental

cost-effectiveness ratios (ICER) were estimated to be 11999 CHF per saved per Quality Adjusted Life Year (QALY) for the strategy Self-HPV/colpo, 10675 CHF per saved QALY for the strategy Self-HPV/Pap and 21108 CHF per saved QALY for the strategy PAP/HPV. Sensitivity analyses demonstrated that the ICER was robust to all parameters.

References

Offering Self-HPV as a CC screening strategy to non-attendees in Switzerland is cost-effective and is associated with a higher reduction of CC cases and related deaths compared to the currently used cytology-based screening.



00432

HEALTH IMPACT OF THE NINE-VALENT HPV VACCINE IN THE NETHERLANDS

32. Economics and modelling

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Background / Objectives

In 2009 the Netherlands have implemented HPV-vaccination in the Dutch National immunisation program (NIP) to prevent cervical cancer as around 700 women are still diagnosed with cervical cancer every year. The 2-valent HPV vaccine is currently used in the NIP. In 2015 the EMA licensed a second generation HPV-vaccine, the 9-valent HPV vaccine (Gardasil 9®) that protects against infections of seven high-risk HPV-types (HPV 16/18/31/33/45/52/58) and two low-risk HPV-types (HPV 6 and 11). This vaccine can prevent around 90% of all HPV-positive cancers, including cervical, vaginal, vulvar, penile, anal and head/neck cancers, and genital warts and around 82% of high-grade precancerous lesions. The aim of this study is to assess the health impact of the nine-valent HPV vaccine on HPV-related cancers, precancerous lesions and genital warts in the Netherlands.

Results

A previously published dynamic transmission model was calibrated on Dutch demographical and epidemiological data to simulate the HPV transmission dynamics and the occurrence of HPV-related diseases in the Netherlands. With the calibrated model predictions of the impact of HPV-vaccination are made over a period of 100 years comparing the status quo of offering the bivalent vaccine to 12 year old girls with a nine-valent vaccine for girls only and for boys and girls. In the two scenarios HPV-vaccine coverage of 53% and a two dose schedule were used to reflect the current HPV-vaccination program.

Conclusion

Replacing the 2-valent HPV vaccine with the 9-valent HPV-vaccine can, compared to the 2-valent vaccine, lead to an additional reduction of the incidence of CIN2/3 with 24.9% and cervical cancer (CC) with 12.5%, preventing 36996 CIN2/3 cases, 5564 CCs and 1409 CC deaths in 100 years. Girl only vaccination can also avert an extra 125 anal cancers (AC) and 20 anal cancer deaths (men and women combined). Protection against HPV-6/11 by the 9-valent vaccine can avoid 1.2 million of genital warts cases.

Extending the Dutch NIP with HPV-vaccination for boys will reduce the number of HPV-related cancers even further. Compared to the current program the 9-valent vaccine can prevent 7869, 5689, 1707, 976, 30 and 21 cervical, head/ neck, anal, penile, vulvar and vaginal cancers, respectively. The reduction of the HPV-related cancers can save almost 4000 deaths in 100 years.

References

The 9-valent HPV-vaccine can have a significant impact on the incidence of HPV-related disease compared to the 2-valent vaccine. Extending the HPV-vaccination program to boys will not only protect the vaccinated, but herd-effects will contribute to a better protection of the opposite sex as well.

00490

A SIMPLIFIED MODEL OF THE COST-EFFECTIVENESS OF SCREENING IN THE R PROGRAMMING LANGUAGE: A TEACHING AND RESEARCH TOOL

32. Economics and modelling

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Background / Objectives

To demonstrate a simplified pedagogical model of the cost-effectiveness of cancer screening and explain its potential as a teaching and research tool. Secondly, to show the model's relevance in the context of cervical cancer screening.

Results

The models applied in cost-effectiveness analyses of screening interventions are typically designed to address specific policy questions and consequently are often large, complex and opaque. We describe the rationale for employing a lightweight, fully shareable and transparent alternative to such applied models for the purposes of teaching and methods research. We present the code of a simplified, discrete-event, microsimulation model of cancer screening coded in the R statistical programme and supported with a Microsoft Excel-based user interface for the specification of input parameters. We demonstrate the components of the model relating to the natural history of disease, test performance and anticipated health gain and healthcare costs.

Conclusion

We show how the costs and effects of multiple alternative screening strategies can be simulated in the model. Using the process of comparative statics, we show how the efficient frontier and incremental cost-effectiveness ratios of alternative screening programmes vary with changes in key parameters such as disease incidence and test sensitivity. Furthermore, we demonstrate how the choice of the optimal screening policy for a given cost-effectiveness threshold varies with changes in input parameters. As such, the model provides a tool with which to demonstrate the qualitative relationships between parameters and the optimal policy in a way that is faster and more accessible than employing a full applied model. The relevance of this

model is demonstrated in the cervical screening context of varying risk groups between vaccinated and unvaccinated women and the choice between cytology and HPV-based testing.

References

The simplified model provides a transparent and easy-to-use demonstration of the fundamentals of the cost-effectiveness of screening. The model is fully shareable and represents a useful open-source teaching and research tool to enhance methods research in the cost-effectiveness of screening. Most models used in applied research are not fully published, due both to their large size and to concerns about sharing intellectual property. Such incomplete reporting compromises transparency and hinders methods research. Our simplified model avoids these problems with fully-shareable code that can be employed and adapted by anybody. This alternative offers a more appropriate tool for teaching the basics of screening cost-effectiveness and conducting methods research.

FC 22. HPV testing + genotyping

00122

Prevalence and genotype distribution of Non HPV-HR types in women with High grade cervical lesions in Northern area in Israel

09. HPV screening

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Background / Objectives

Invasive cervical cancer is caused by human papillomavirus (HPV). This study describes the prevalence and genotype distribution of low risk (LR) HPV types in women with high grade cervical lesions and cervical cancer.

Results

The study summarized HPV types detected in 6654 samples which were sent to the Serology laboratory from cervical clinics in northern Israel during the years 2006-2017. Four hundred and one women were diagnosed with CIN 2-3, and 205 with cervical cancer. The HPV test was performed during investigation of ASCUS (Atypical Squamous Cells of Undetermined Significance) results on Pap test or due to complaints suggestive of cervical neoplasia. Twelve HPV types were classified as high risk (HPV-HR) and the other Non HPV–HR types 40,42,53,54,56,62,67,70,73,81,82. (Non HPV-HR).

Conclusion

HPV-LR types were detected in 7.3% (28/379), and 4.8% (9/189) of women with Cervical Intraepithelial Neoplasia (CIN) 2-3, and women with cancer respectively. HPV was negative in 5.4 % (22/401) and 7.8 % (16/205) of women with CIN 2-3 and cervical cancer and respectively.

References

More data should be collected in order to decide if HPV screening should include more HPV types in order to improve detection rate of CIN 2-3 and cervical cancer.

Detection of Non HR-HPV types will be more important in the future after HPV vaccine which will decrease the prevalence of HPV 16 and 18.

00092

Identifying the causal HPV genotypes in high-grade cervical lesions using HPV genotyping of cervical screening samples

11. Genotyping

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Background / Objectives

To assess the clinical impact of HPV vaccination, the causal HPV genotypes in CIN2+ must be identified. The hierarchical and proportional methods are widely used for this purpose, but these ignore the occurrence of multiple lesions and do not adjust for the genotype distribution in the general population. We aim to develop a new method for identifying the causal HPV genotypes in CIN2+ based on cervical screening samples.

Results

Our model assumes that women may have multiple lesions caused by different HPV genotypes and that HPV genotypes have independent CIN2+progression risks. We applied our method to 512 women with abnormal cytology who tested positive for at least one of the 25 HPV genotypes detected by SPF10-LiPA. We validated our method by means of laser-capture microscopy (LCM)-polymerase chain reaction (PCR).

Conclusion

We predicted 274 type-specific lesion where 280 type-specific lesions, in 252 women, were observed by LCM. HPV16 and HPV33 had the highest estimated CIN2+ progression risk: 68% (95%CI: 61 – 75%) and 64% (46 – 83%), respectively. All low-risk HPV genotypes had negligible risk (i.e., <1%) of CIN2+. The genotype attributable fractions (AFs) estimated by our method were closer to the AFs observed by LCM-PCR than those estimated by the proportional and hierarchical methods. HPV16 and HPV31 were estimated to attribute the most: 0.47 and 0.15, respectively.

References

Our new method estimates HPV genotype attribution in cervical lesions accurately without prior assumptions about type-specific oncogenicity. This method can play an important role in monitoring HPV vaccine effectiveness.

00239

THE ROLE OF HPV GENOTYPING IN POST-TREATMENT FOLLOW-UP OF CERVICAL INTRAEPITHELIAL NEOPLASIA.

11. Genotyping

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Background / Objectives

Recurrences of cervical intraepithelial neoplasia (CIN) may occur in approximately 5-15% of cases within 2 years after conservative surgical treatment. Although many patient-related factors (age, smoking, number of sexual partners) and pathological characteristics of CIN (histological grade, glandular involvement and surgical margins) may affect the risk of recurrence, persistent positivity of HPV testing has been widely identified as the main predictor for the development of disease relapse. The aim of this study was to investigate the role of HPV genotyping for selecting women at high-risk of recurrence.

Results

Women undergoing surgical treatment of CIN, who performed HPV genotyping at baseline and the first follow-up visit, at the European Institute of Oncology, Milan, from January 2003 to December 2014, were selected for a retrospective analysis. HPV genotype was detected by the Roche Diagnostics Linear Array test on liquid-based cervical samples. HPV persistence was defined as the detection of at least one genotype at baseline that was still present after 6 ± 3 months. Relapse-free survival and the 2-years cumulative incidence were estimated by using the Cox and the Gray's models, covariates were infections status, baseline histology, age and HPV genotype.

Conclusion

Among 408 patients enrolled, HPV-persistence was shown in 89 women at the first follow-up. No significant difference was proven between HPV-not persistent and HPV persistent groups, according to age and baseline histology. Multiple infections were significantly associated to HPV-persistent patients (p=0.003). Overall, 26 relapses occurred, with a 2-years cumulative incidence of 10.6 (95% CI=6.7-15.6). HPV persistence (HR=6.2, 95% CI=2.7-13.8 and 2-years cumulative incidence of 26.1, 95% CI=14.1-39.7), multiple genotypes infection (HR=6.6, 95% CI=2.2-19.8 and 2-years cumulative incidence of 31.8, 95% CI=8.6-58.6) and the presence of HPV 16/18 with/without other high-risk genotypes (HR=8.3, 95% CI=3.6-18.8 and 2-years cumulative incidence of 31.3, 95% CI=16.7-47.1) were significantly associated to higher risk of relapse (p<.001). HPV 16 (60.0%) and HPV 18 (10%) were the most prevalent genotypes at follow-up, even if at baseline HPV 16 was the most frequent (33.0%) but followed by HPV 31 (26.3%) and HPV 58 (10.3%), whereas HPV 18 was not common (3.0%).

References

The detection of the same HPV-genotype at 6 months is a relevant predictor of recurrence. Moreover, persistence of HPV 16/18 has a significant impact on relapse-free survival. Therefore, HPV genotyping could be useful for a better risk stratification in post-treatment follow-up of CIN.

00242

SYSTEMATIC LITERATURE REVIEW ON TRIAGE STRATEGIES FOR HPV POSITIVE AND ASCUS/LSIL PATIENTS: ROLE OF EXTENDED HPV GENOTYPING VS OTHER TRIAGE METHODS

11. Genotyping

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Background / Objectives

Objectives: Advances in the screening and detection of cervical disease have been greatly aided by the inclusion of HPV testing along with cytology to identify patients at risk for CIN2+ disease. Various triage methods have been described in the literature to improve patient referral to colposcopy from HPV positive patients, as well as ASCUS and LSIL cases.

Results

Methods: We undertook a systematic review of literature to compare relative effectiveness of these triage methods. The analysis included PubMed, PubMed Central, the Database for Abstracts of Reviews of Effects, and the Cochran Database of Systematic Reviews from 2000 through 2017 for relevant controlled clinical trials and observational studies. In addition, a supplemental review was conducted by searching retrieved article references. Metrics of clinical effectiveness included incident detection of CIN2+ and colposcopy referral rates.

Conclusion

Results: 1281 articles were initially identified by the various search strategies. 255 articles were screened for inclusion/exclusion criteria and 45 articles were retrieved for data review and comparison. Finally, 20 articles were selected for data analysis and summary. Primary screening methods included cervical cytology and high risk HPV (hrHPV) testing. Triage methods evaluated in this systematic literature review included: (i) protein biomarker assays (e.g., immunocytochemistry with p16 and Ki-67; HPV E6 and E7 protein detection); (ii) HPV mRNA testing; (iii) DNA methylation markers; and (iv) HPV extended genotyping.

References

Conclusions: Relative to cytology-based triage of high-risk HPV positive patients, all the studied triage methods displayed varying degrees of utility in the detection of incident CIN2+ disease. HPV mRNA, protein biomarkers and HPV extended genotyping improved the detection of CIN2+ within ASCUS and LSIL cases. Protein biomarkers and DNA methylation represented an alternative to cytology in the triage of HPV positive patients. Protein biomarkers, DNA methylation and HPV extended genotyping displayed comparative negative predictive value over a 3-year follow-up period. In addition, the use of extended HPV genotyping permitted stratification of patients for immediate referral to colposcopy based upon increased risk for CIN2+ (HPV 16, 18, 31, 33) versus delayed colposcopy with re-testing at 1 year follow-up for certain HPV genotypes (e.g., 51, 56, 58, 59, 66). Protein biomarkers and extended HPV genotyping also reported reductions in colposcopy referrals. Additional comparative clinical studies appear warranted to directly compare these various triage methods within the same patient cohort for clinical utility and cost effectiveness.

00335

CLINICAL VALIDATION OF THE FULL GENOTYPING CLART4S HPV ASSAY ON SUREPATH COLLECTED SCREENING SAMPLES ACCORDING TO THE INTERNATIONAL GUIDELINES FOR HUMAN PAPILLOMAVIRUS TEST REQUIREMENTS FOR CERVICAL SCREENING

11. Genotyping

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Background / Objectives

“additional contribution”

Novel HPV assays intended for cervical screening use must be evaluated in accordance with International guidelines for Human Papilloma Virus test requirements for cervical cancer screening. The CLART HPV4S assay (CLART4S, Genomica, Madrid, Spain) is a PCR based microarray assay targeting the L1 region, and the first full-genotyping assay to detect oncogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and three non-oncogenic-HPV genotypes (6, 11, 66) to achieve fulfillment of international validation criteria using SurePath screening samples. Here we present the outcome of the validation of this novel full-genotyping assay on SurePath collected screening samples, using the GP5+/6+ PCR assay (GP5/6) as a comparator/reference. The genotype concordance between CLART4S and GP5/6 was also assessed.

Results

To assess the performance of the CLART4S assay, SurePath screening samples from women 30 years and above participating in the Danish cervical cancer screening program were collected at Copenhagen University hospital, Hvidovre. For the clinical sensitivity analysis, 81 samples from women with confirmed CIN2 or greater were collected. For the clinical specificity analysis, 1184 samples from

women with less than CIN2 histology were collected. The assay results were compared to that of the GP5/6 assay in collaboration with Karolinska Institutet, Stockholm. The laboratory performance element involved testing 540 individual samples with known GP5/6 results. The inter-laboratory agreement was performed in collaboration with the Scottish HPV Reference Laboratory in Edinburgh, Scotland.

Conclusion

The relative sensitivity of CLART4S was 91.3% (GP5/6=92.6%) and relative specificity was 90.7% (GP5/6=91.0%). The CLART4S assay was shown to be non-inferior to that of GP5/6 for both sensitivity (p=0.00) and specificity (p=0.02). The genotype specific concordance between CLART4S and GP5/6 was good for 12 oncogenic HPV types. The Kappa value for intra-laboratory reproducibility was 0.84 (lower confidence bound 0.92) and for the inter-laboratory agreement the kappa value was 0.72 (lower confidence bound 0.87).

References

This is the first report on the clinical validation study of a full-genotyping HPV assay applied to SurePath collected samples. Using GP5/6 as comparator, the CLART4S performed well and met the International guidelines for sensitivity, specificity, intra-laboratory reproducibility and inter-laboratory agreement. The CLART HPV4S assay is therefore a good candidate for use in cervical cancer screening programs, especially programs utilizing genotype information in the screening algorithms.

00399

Comparison of partial HPV genotyping using the Cobas 4800 HPV test and the Aptima HPV 16 18/45 Genotype assay

11. Genotyping

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Background / Objectives

Partial HPV genotyping has the potential to further stratify HPV positive women in HPV primary screening. This study compares two partial HPV genotyping approaches, the Cobas 4800 HPV test for detection of HPV 16 and 18 and the Aptima HPV genotype assay for detection of HPV 16 and 18/45.

Results

In partnership with CervicalCheck, The National Cervical Screening programme, CERVIVA are undertaking a longitudinal observational HPV primary screening study which is evaluating 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 mRNA-positive women are further tested with the Aptima HPV 16 18/45 genotype assay for detection of HPV16 and 18/45. The performance of different genotyping strategies is being examined both cross-sectionally and longitudinally over two screening rounds for detection of CIN2+.

Conclusion

In total, 9853 primary screening samples were tested for HPV DNA and mRNA. Overall, 4.8% [469/9853] tested positive for HPV 16/18 DNA and 3.6% [357/9853] for HPV 16/18/45 mRNA. There was good agreement between both assays for detection of HPV 16/18 and HPV 16/18/45 (97.67%, Kappa co-efficient: 0.678). The HPV 16/18

DNA positivity rate was significantly higher than HPV 16/18/45 mRNA positivity rate in women who were normal on cytology [2.9% vs 1.7% p=<0.001]. There was no significant difference in the rate of positivity in women with LSIL/ASCUS [18.4% vs 17.5% p=0.643] and HSIL [62.9% vs 58.6% p=0.211] between the Cobas HPV test and the Aptima HPV 16 18/45 genotype assay respectively. The Linear Array HPV genotyping test (Roche) was used elucidate a subset of discordant samples.

References

Overall there was good agreement across both assays. The Aptima HPV 16 18/45 genotype assay had fewer positives in women who were normal on cytology. Longitudinal follow up data will determine and compare the clinical performance of each genotyping assay for detection of high grade cervical neoplasia.

00088

ASSESSMENT OF ATTRIBUTION ALGORITHMS FOR RESOLVING CIN3-RELATED HPV GENOTYPE PREVALENCE IN MIXED-GENOTYPE BIOPSY SPECIMENS USING LASER CAPTURE MICRODISSECTION AS THE REFERENCE STANDARD

19. New technologies

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Background / Objectives

Determining the single causative HPV genotype of each high-grade cervical lesion and/or cancer is an important measure of vaccine effectiveness in preventing vaccine-type-specific disease. Laser capture microdissection (LCM) and genotyping of lesions is considered the gold standard: however, it is resource-intensive and many large studies use easier-to-collect samples and mathematical algorithms to attribute genotype where multiple genotypes are detected. To date, these algorithms have not been assessed against LCM genotyping.

Results

Cervical biopsy specimens (n=531) containing cervical intraepithelial neoplasia grade 3 (CIN3) lesions were genotyped as whole tissue sections (WTS) (RHA kit HPV SPF10-LiPA25, v1.0 and SPF+ strips). LCM, and proportional, hierarchical, single type only and maximum (any type) attribution methods were used to resolve mixed genotype detections. LCM was also used to re-test any samples that were negative for high-risk-HPV genotypes.

Conclusion

Figure 1. Comparison of nonavalent HPV genotype prevalences generated by different methods to resolve mixed-genotype detections in CIN3 biopsy specimens.Of 531 specimens, 14 were excluded from analysis (13 invalid DNA results, 1 could not

be resolved to a single genotype using LCM), leaving 517. Of these, mixed-genotype detection occurred in 71 (13.7%) of WTS. The results of the 5 attribution methods are shown in Figure 1for nonavalent vaccine genotypes. There were no statistically significant differences between proportions of each genotype by attribution method, although proportional attribution provided the lowest genotype-specific prevalence estimates overall.

References

In CIN3 biopsy specimens, including mixed-genotype detections, attribution algorithms to resolve mixed infections to a single causative genotype gave comparable results to the reference method LCM.

FC 23. Molecular markers 2

00199

GROWTH POTENTIAL AND APOPTOSIS IS INHIBITED BY LOCALISED TOPICAL MICROWAVE ENERGY IN HPV16-POSITIVE CERVICAL TUMOUR CELLS IN 3D TISSUE CULTURE MODELS

01. Viral and molecular biology

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Background / Objectives

Raising the temperature of tissues into the “fever” range of 38-45°C as a therapeutic is known as hyperthermia. Localised hyperthermia has been used previously as an adjuvant to cancer therapies [1]. In the case of benign lesions, microwave treatment of HPV-positive verrucas resulted in 75.9% clearance of lesions [2]. We characterised the molecular effects of microwave treatment on in vitro cultured HPV-16 positive cervical cancer tissues as a potential new therapy for HPV-associated disease.

Results

The medical device, “Swift” (Emblation Ltd, UK) delivers microwaves through a 7mm contact site and is currently used in the clinic for the treatment of verrucas. The delivery of the microwaves to tissues results in heating to around 48^{o}C and causes apoptosis of nearby cells. SiHa cells containing the integrated HPV16 genome were grown on organotypic raft cultures and treated with the Swift device. Immunohistochemistry and immunofluorescence microscopy (antibodies against HPV E6, p53, cleaved caspase 3, Ki67, MCM2, HSP70, G3BP) was used to determine the molecular effects of the device. Quantitative PCR was used to measure expression of immune modulators.

Conclusion

Microwaving induced apoptosis at the treatment site alongside a reduction in cellular growth/proliferation. HSP70 expression was observed in regions of the rafts treated with the microwave device, confirming hyperthermia. The levels of the HPV E6 oncoprotein were downregulated by the treatment and levels of p53 were upregulated in a sustained manner in tissue areas adjacent to the treatment site.

Microwaving induced translational stress but did not induce conventional inflammatory pathways.

References

Current treatments for anogenital pre-cancers and warts are painful and invasive. We aimed to characterise the effects of microwaving on HPV-infected tissue as a potential less invasive method of eliminating disease. The delivery of microwaves via the Swift probe, allows the natural apoptosis of HPV-infected cells to resume. Therefore, the Swift probe presents as a promising new, less painful treatment for the elimination of HPV-infected cells in anogenital lesions.

References

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00237

K14-HPV16 MOUSE MODEL: A JOURNEY TOWARDS EARLY HPV-INDUCED HEAD AND NECK VS ANAL AND UTERINE CARCINOGENESIS

01. Viral and molecular biology

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Background / Objectives

Human Papillomavirus (HPV) is the most common sexual transmitted agent worldwide, being also responsible for 5 % of all human cancers. Even though cervical cancer is thought to be reducing, HPV positive anogenital and head and neck cancers are regrettably increasing. Differences in the natural history of HPV have been observed by gender and anatomic sites of infection where epithelial backgrounds and tissue microenvironment may play a crucial role. The main goal of this study was to understand if E6, E7 and E5 oncoproteins may promote distinct roles in the carcinogenesis cascade in K14-HPV16 mice model base of tongue, anus and uterine cervix characterized by different tissue microenvironments.

Results

The base of the tongue, anus and uterine cervix samples were collected from 10 female and 10 male 30-week-old K14-HPV16 transgenic mice. Histopathological analysis of the tissues was performed for tissue characterization. Tissue samples were classified as normal, hyperplasic, dysplastic and carcinoma. The E6, E7 and E5 mRNA levels were quantified by real-time PCR being normalized by a combination of the best two housekeeping genes. Statistical analysis was performed using the IBM SPSS Statistics (Version 24.0). Mann-Whitney tests were used to evaluate statistical differences in normalized relative expression (–ΔCt) of the E6, E7 and E5 genes among the different tissue samples.

Conclusion

We observed a higher incidence of more advanced lesions, namely dysplasia and carcinoma on the base of the tongue tissue samples in comparison with the uterus and anus where all lesions were hyperplasic. The expression of the oncogenic HPV viral mRNA was detected across tissues with no significant overexpression within the different lesions.

References

This study enlightens the proof of concept of an earlier and less-cofactor dependent carcinogenesis induced by HPV in oropharyngeal cancers in comparison with other anatomic localizations. In the base of the tongue, cancer was induced within the mice 30 weeks period, in comparison with the anus and uterine cervix, where HPV itself seems not to be sufficient to promote advanced lesions even though the expression of the viral mRNA´s are detected and similar within the tissues. Future studies should focus on understanding the behavior of the HPV oncoproteins and the related oncogenic pathways at multiple anatomic locations of infection, representing different tissue microenvironments. This might allow a better understanding of tissue-specific HPV-related carcinogenic steps and a consequent precision therapy.

00250

HUMAN PAPILLOMAVIRUS (HPV) DNA DETECTION IN PLASMA AND IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) SAMPLES OF WOMEN WITH A RECENT HISTORY OF CERVICAL DYSPLASIA

01. Viral and molecular biology

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Background / Objectives

The presence of viral DNA has been suggested to represent a marker for viral associated cancers. Some studies have reported the presence of HPV DNA in the bloodstream of women with cervical cancer, indicating the possible circulation of HPV-associated cancer cells. On the contrary, the presence of HPV DNA in blood of women with precancerous lesions has been less frequently reported. The aim of this pilot study is to investigate the presence of HPV DNA in cervical, plasma and PBMCs samples of women with a recent history of cervical dysplasia.

Results

Paired blood and cervical samples have been collected from 53 women referred for colposcopy at San Gerardo Hospital, Monza, Italy. Nucleic acids extraction was performed using NucliSENS easyMAG (bioMérieux). HPV detection in cervical samples was assessed by real-time PCR using AnyplexII™ HPV28 (Seegene). HPV 16, 18, 31, 33, 45, 51 and 52 DNA detection on plasma and PBMCs was performed using highly sensitive quantitative "in house" genotype specific real-time PCR assays. Genotype-specific oncogenic transcripts detection was assessed by “in house” real-time RT-PCR assays using the iTaq™ Universal SYBR ® Green One-Step Kit.

Conclusion

One or more HPV types were detected in 83% (44/53) of cervical samples, with HPV16 and HPV31 resulting the most prevalent genotypes. Seven of the studied women (7/53; 13.2%) were found to be HPV DNA positive in plasma samples.

HPV16 resulted the most prevalent genotype in plasma, with an average viral load of 336 copies/ml. Of these, only one woman was shown to have the same genotype in both cervical and plasma samples. Preliminary results of HPV DNA detection in PBMCs have shown a positivity of 4%, 2/53 (HPV16 in both cases), with a viral load of 6.87E+01 copies/10^5 cells and 1.32E+01 copies/10^5 cells respectively. One of these two samples was also positive for the presence of HPV16 oncogenic transcripts.

References

These preliminary results confirm that HPV DNA can be detected in peripheral blood samples of women with a recent history of cervical dysplasia and that oncogenic transcripts can be identified in PBMCs. Further studies are required to evaluate the significance of the presence of high-risk HPV DNA and RNA in the bloodstream of women with early stages of cervical dysplasia.

00218

mRNA biomarker detection in liquid-based cytology: a new approach in the prevention of anal cancer

12. Molecular markers

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Background / Objectives

Anal cancer (AC) incidence has increased in certain populations such men who have sex with men (MSM), HIV-positive individuals and women with high-risk human papillomavirus (hrHPV) infection.

hrHPV is considered the main etiological agent of AC, leading premalignant lesions (high-grade intraepithelial lesion/anal intraepithelial neoplasia grade 2-3 [HSIL/AIN2-3]). Current literature suggests that screening for anal HSIL/AIN2-3 should be considered in high-risk groups using liquid-based cytology (LBC) and high-resolution anoscopy (HRA). However, the success of this strategy has not been proved. Other studies have shown that hrHPV testing and triaging with molecular markers might be an approach for AC screening.

We aimed to determine the feasibility of the detection mRNA expression of CDKN2A/p16, MKi67 and TOP2A in anal LBC to evaluate whether these biomarkers might be useful in the identification of patients with HSIL/AIN2-3.

Results

We included 125 MSM positive for HIV (MSM-HIV) referred to the AC unit of our center during 2016. At the initial visit, patients undergo anal LBC, hrHPV testing, HRA and at least one biopsy. We selected MSM-HIV with cyto-histological concordant results in the first visit. MSM-HIV included in the study were grouped into three categories: control group (men with a negative Pap test, biopsy and hrHPV testing; n= 77), low-grade SIL group (LSIL; patients with a Pap test and biopsy showing LSIL and positive hrHPV testing; n= 28), and HSIL group (men with Pap test and biopsy of HSIL and positive hrHPV testing; n= 20). After RNA extraction (RNeasy RNA extraction kit; Qiagen), the expression of CDKN2A/p16, MKI67 and TOP2A was analyzed by reverse transcription and quantitative PCR in LBC. The data were analyzed with SPSS Version 19.0.

Conclusion

Mean normalized ΔCycle threshold (ΔCt) of the different biomarkers for the control, LSIL and HSIL group did not differ significantly. For TOP2A, ΔCt (95% confidence interval [CI]) values were 64.8 (13.6-116.1); 44.4 (21.2-67.6) and 19.4 (9.8-29.1), respectively; p 0.587. For MKi67 ΔCt (95% CI) values were 101.0 (0.1-202.5); 42.9 (14.8-71.1) and 13.2(5.7-20.6), respectively; p= 0.535. For CDKN2A ΔCt (95% CI) values were 3.5 (2.4-4.6), 3.0 (1.7-4.2) and 1.0 (0.6-1.3), respectively, for the different diagnostic categories; p=0.062.

The area under the ROC Curve (95% CI) for CDKN2A/p16, MKI67 and TOP2A were 0.74 (0.66-0.82), 0.73 (0.64-0.80) and 0.60 (0.51-0.69), respectively.

References

mRNA detection in anal LBC specimens is feasible. Further studies including a larger number of patients are warranted to confirm that biomarker identification using mRNA-based strategies might have a role in the secondary prevention of AC.

00255

Identification of productive and transforming cervical and anal intraepithelial neoplasia using immunohistochemical markers p16INK4a and HPV E4

12. Molecular markers

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Background / Objectives

To improve diagnosis and identify lesions needing treatment, biomarkers that show distinct expression patterns between transforming and productive high-grade squamous intraepithelial lesions (HSIL) are needed. We investigated the expression of immunohistochemical biomarkers HPV E4, a marker of productive HPV infection, and $p16^{INK4a}$, a marker of transforming HPV infection, in cervical and anal intraepithelial neoplasia (CIN and AIN).

Results

A reference grading was established using expert, consensus, subjective HE diagnoses supported by p16 expression for 243 cervical and 183 anal biopsies. Worst lesions were scored for p16 (0-4), identifying transforming infections and HPV E4 (0-2), marking the productive aspect of the lesion.

Conclusion

Reference grading resulted in: 78 CIN0, 46 CIN1, 37 CIN2, 82 CIN3, 37 AIN0, 67 AIN1, 43 AIN2, 36 AIN3. In both CIN and AIN, proportion of diffuse p16 positive lesions increased with the severity of the lesion (100% of CIN2 and CIN3, 91% of AIN2 and 97% of AIN3). Significantly more AIN1 lesions showed extensive patchy p16 staining compared to CIN1 ($p<0.001$). HPV E4 was positive in very similar

proportions of CIN0 and AIN0 (0% and 0%), CIN1 and AIN1 (46% and 49%), CIN2 and AIN2 (49% and 56%) and CIN3 and AIN3 (6% and 3%).

References

Combined p16/E4 staining can provide detailed information beyond supporting H/E classification, that could potentially allow more selective treatment of HSIL. Differences in p16 expression between CIN and AIN might relate to differences in site, involvement of HIV and progression which need to be considered in managing screen-detected lesions.