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HPV16 MINORITY VARIANTS AMONG CERVICAL AND ANAL SAMPLES WITH SINGLE HPV16 OR MULTIPLE HPV TYPES INFECTIONS


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Background / Objectives

To assess presence of minority variants (MV) in HPV16 genomes isolated from clinical cervical and anal specimens of patients followed in Paris area, France, using ultra-deep sequencing of the whole viral genome.

Methods

HPV detection and typing was performed with Anyplex®II HPV28 detection kit (Seegene). Ultra-deep sequencing was performed using Illumina® platform. HPV16 lineages were determined by phylogenetic analysis using RAxML. HPV16 viral loads were determined by “in house” real-time PCR assays. Viral genome integration ratio was assessed by E2/E6 ratio.

Results

We assessed 44 consecutive smears routine samples (28 cervical and 16 anal [n=12 men]) positive for HPV16. 19 patients (43%) were HIV-infected. Cytohistologic data showed 10 LSIL and 5 HSIL in cervical samples, 3 LSIL and 8 HSIL in anal samples. Overall, multiple HPV (mHPV) infections (high risk [hr] and/or low risk [lr]) were observed in 32 samples (73%). In anal samples mHPV infections were present in a higher proportion (94% vs 61%, p=0.03) and with a higher diversity of hrHPV types (p=0.04) than in cervical samples. Phylogenetic analysis showed diversity in HPV16 lineage variants with 32 A lineage (73%), 3 B (7%), 7 C (16%) and 2 D (4%). Ultra-deep sequencing analysis revealed that nucleotidic non synonymous substitutions
were present in minority proportion (i.e. ranging from 2 to 20%) in 14 samples (32%), without difference between cervical and anal samples (32% and 31%, respectively). Viral MV were present at the median proportion of 3.4% (IQR=2.4-6.4). Most of MV (64%) presented only a single nucleotide variation on the whole genome, located in E1 or E2 regions in most of cases (86%). Among cervical specimens, a lower frequency of lrHPV coinfections was observed in samples displaying MV than in samples without MV (11% vs 58%, p=0.04). A lower frequency of hrHPV coinfections was also observed but was no significant (33% vs 63%). In addition, patients with mHPV cervical infections had more frequently high HPV16 viral load (i.e. >50 copies/cell) than patients without mHPV infections (77% vs 20%, p=0.012). The proportion of patients with a high integrated viral genome ratio (i.e. >50%) increased with the cervical lesion grade: 44%, 56% and 80% in ASC-US, LSIL and HSIL, respectively. Regarding anal samples, all except one had mHPV infections, 50% were HSIL and 55% had a high integrated viral genome ratio.

Conclusion

This first large study of HPV16 whole genome ultra-deep sequencing showed presence of viral MV in one third of the samples, in cervical as well as in anal specimens. In addition, we evidenced that, in almost all cases, cervical samples with MV had no lrHPV coinfections.
DETECTION OF CERVICAL HUMAN PAPILLOMAVIRUS IN WOMEN ATTENDING CERVICAL CANCER SCREENING BY VISUAL INSPECTION IN COTE D’IVOIRE


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Background / Objectives

Human papillomaviruses (HPV) cause precancerous lesions and cancers of the cervix. In Côte d’Ivoire, cervical cancer screening program based on visual inspection is the gold standard. This study aims to detect High risk (HR) HPV DNA on women attending for cervical cancer screening program based on visual inspection after application of acid acetic then lugol

Methods

From March to December 2015, endocervical samples from women attending cervical screening were tested for some HR-HPV. HPV DNA was amplified using PGMY09/11 primers which generated 450 base pairs at the L1 region. The samples harboring HPV DNA were genotyped using the multiplex PCR with HPV 16, 18, 31, 33, 35, 45 and 51 primers.

Results

The mean age of this population was 32 years. On 339 women enrolled on visual inspection 6.19% were positive. HPV DNA was obtained in 9.73 of the population. Thirty-one of 33 samples (93.93.%) of HPV DNA+ were genotyped using multiplex PCR testing for HPV 16,18, 31, 33, 35, 45 and 51 Of those women with HPV DNA+. 28.57% had a single infection while 71.43% had a multiple infection. HPV genotypes prevalence were the followed: HPV 16 (30.00%), HPV 18 (25.00%), HPV35 (20.00%), HPV 45 (20.00%), HPV 51 (3.30%) and HPV 33 (1.60%). By using PCR as gold standard VIA sensitivity was 16.12% and specificity 95.45 %

Conclusion

HPV prophylactic vaccine would prevent 33.33% of HR HPV infection with the 2v, 33.33% with the 4v and 66.66 % with the 9v vaccines respectively. In Cote d'Ivoire screening for cervical cancer with HR HPV testing and triaging for treatment with
visual inspection would represent a very efficient prevention of cervical cancer program.

References


P02-01
DETECTION OF HIGH-RISK HPV DNA IN CHAGASIC MEGAESOPHAGUS WITH AND WITHOUT CANCER

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Background / Objectives

Esophageal cancer (EC) is the eighth most common type of cancer worldwide frequently found with esophageal squamous cell carcinoma (ESCC) differentiation. The main risk factors related to ESCC development are smoking, alcohol consumption, chagasic megaesophagus (CME) (common digestive chronic manifestation of Chagas Disease), and likely HPV, although the role of HPV in ESCC carcinogenesis is still disputable. Therefore, the present study aimed to detect high-risk HPV DNA in patients with chagasic megaesophagus with and without cancer and to correlate these findings with clinicopathological data.

Methods

Samples tissue/biopsy specimens fixed paraffin were retrospectively collected from the southeast region of Brazil obtained from patients treated in two hospitals: Universidade Federal do Triangulo Mineiro e Barretos Cancer Hospital. Cases were divided in two groups: CME (n=30) and ESCC/CME (n=21). The detection, and typing of high-risk HPV were performed by multiplex PCR (Luminex).

Results

Overall, the prevalence of HPV was higher in the CME group (n=15/30, 50%) when compared to the ESCC/CME group (n=8/21, 38%). Among the HPV types detected, HPV-16 and HPV-73 (13.3% and 6.7%, respectively) were detected with similar frequency in both groups. On the other, HPV-45, HPV-51 and HPV-56 (6.7%, 10% and 6.7%, respectively) were found only in the CME group, as well low-risk HPV-6 and HPV-11 (3.3% and 3.3%, respectively). In addition, HPV-positive patients (38.1%) had I/II (non-advanced) grade megaesophagus, whereas HPV-negative patients (61.9%) had grade III/IV (advanced), staging T3/T4 (91.7%), N0/N1 (91.7%) and M0 (75%). Regarding the pathological features of the ESCC/CME group, the most frequent type reported was moderately differentiated, which was more...
frequently associated with HPV-negative status, yet not reaching statistical significance.

Conclusion

Despite the high frequency of HPV DNA detected in patients with chagasic megaesophagus with and without cancer, a statistically significant association was not found, thus further studies are important in order to understand the role of HPV in esophageal cancer in patients with megaesophagus.
Comparative study of HPV prevalence in glans and urine between the patients with prostate cancer and benign prostatic hyperplasia


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Background / Objectives

Some recent studies demonstrate that the urinary tract is alternative common site for HPV infection in men. Although urine is often used for investigating HPV infection in the urinary tract, a significance of HPV detected from urine samples has been not understood. We investigated the prevalence of human papillomavirus (HPV) in the genital and urinary tract among the patients with prostate cancer (Group A), ones with an elevated level of prostate specific antigen (PSA) with no evidence of prostate cancer (Group B), and ones with benign prostatic hyperplasia (BPH) without an elevated PSA (Group C). We compared HPV prevalence in glans and urine samples between the groups.

Methods

A total of 325 patients (42 cases in group A, 84 in group B, and 199 in group C) were enrolled in this study. Rubbed cells samples of glans and urine ones were collected from each patient, and sediment cells were preserved in liquid-based cytology solution, respectively. The β-globin gene was first amplified to confirm the adequacy of the extracted DNA in all samples. HPV-DNA and genotype was determined using GENOSEARCH-31 (BML, Co., Ltd., Nagoya, Japan).

Results

Mean age in group A, B, and C was 77.1, 68.2, and 72.7 years, respectively. Among the adequate samples, HPV was detected in 14.6% (6 cases), 16.6% (11 cases), and 26.8% (47 cases) of glans samples in group A, B, and C. HPV prevalence was higher in group C, but there were no significant differences between the groups. On the other hand, HPV prevalence in urine samples of group A, B, and C, was 12.5% (5 cases), 3.6% (3 cases), and 7.1% (13 cases), respectively. High-risk HPV was identified in 7.5% (3 cases) in group A, 3.6% (one case) in group B, and 3.8% in group C. In urine samples, there were also no significant differences in HPV prevalence between the groups, while high-risk HPV showed a significant higher prevalent in group A than in group B and C (p<0.05).

Conclusion

We found a higher prevalence in urine samples of the patients with prostate cancer, suggesting an interesting issue whether HPV infection in urinary tract can play any
roles in pathogenesis for men, especially in the development of tumors in the urinary tract.
HPV PREVALENCE 10 YEARS AFTER VACCINE INTRODUCTION IN GERMANY – DESIGN OF A POPULATION-BASED STUDY IN 20-25 YEAR-OLD WOMEN

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Background / Objectives

In Germany approx. 4,600 women are diagnosed every year with cervical cancer, leading to 1,500 deaths. Infections with sexually transmitted human papillomaviruses (HPV) are a prerequisite for cervical cancer. Since 2007, routine HPV vaccination for girls has been recommended in Germany. However, because HPV infections are not notifiable in Germany, other means of data collection are needed to evaluate and document the impact of HPV vaccination. In 2010-12, we conducted an initial population-based study among women aged 20-25 years to assess baseline HPV prevalence and genotype-distribution in a mainly vaccine-naïve population. To estimate HPV prevalence ten years after vaccine introduction and to assess possible effects of vaccination, a follow-up study will be carried out in 2017/18. Here we present details of the study design.

Methods

Nationwide population-based cross-sectional study to examine HPV prevalence in 20-25 year-old vaccinated and unvaccinated women in Germany. Cervico-vaginal self-sampling via EvalynBrush (Rovers, Netherlands) and multiplexed genotyping HPV test Optiplex (Diamex, Germany) is used.

Results

We will recruit at least 1,173 women aged 20-25 years. Recruitment will be based on a random sample from the residents’ registration offices of all communities in Germany, using a two-step sampling design stratified by geographical location (former Eastern/Western Germany) and population density (rural/urban). Using a self-sampling device participants will take vaginal cell samples that will be tested for HPV infections with 18 high-risk (e.g. 16, 18, 31, 45) and 8 low-risk types (e.g. 6, 11). In addition, participants will be asked to answer a questionnaire comprising questions on socio-demographics, sexual behavior, immunosuppressive diseases/medication, and HPV vaccination status; furthermore, they will be asked to provide proof of their vaccination status by uploading a photo of their vaccination card. The study was designed by considering major aspects of the baseline study to allow for comparison of results. First results of the study are expected in 2018.

Conclusion
The study will help to answer the following questions:

For women aged 20-25 years in Germany,

What is the prevalence of a) vaccine-preventable and non-vaccine-preventable and b) high-risk HPV types in 2017/18 and in comparison to the baseline study?
What is the difference in HPV prevalence in vaccinated vs non-vaccinated young women?
What is the effectiveness (at population level) of HPV vaccination?
What are risk factors for being unvaccinated or HPV positive?

The study will contribute to the evaluation of the existing HPV vaccination recommendation in Germany.
Trends in rates of treated RRP before and after HPV vaccination among New York children

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Background / Objectives

Recurrent respiratory papillomatosis (RRP) is a rare condition in children acquired around the time of delivery and is caused by HPV types 6 and 11 leading to wart-like growths in the respiratory tract resulting in hoarseness and airway obstruction. Its incidence in children is expected to decline with HPV vaccination which has been FDA-approved in the US since 2006. New York is a populous state with vaccination rates among girls typical of many states nationally, growing from percentages in the 50's to the 60's over the last decade (1). We expect changes in RRP incidence attributable to vaccination to first be evident in young children. We sought to determine whether the rate or frequency of treatments for RRP has changed since the approval of HPV vaccination.

Methods

We obtained data from NY State Ambulatory Surgery and Services Database (2) between years 2004-2013 for discharge encounters of children under the age of 18 who had ambulatory procedures with ICD-9 code corresponding to benign neoplasm of the larynx and procedure codes relating to typical RRP treatments. A visit linkage variable allowed for tracking patients who had multiple discharges in a year. All facilities licensed to perform same-day surgery in NY were included (as such, parameters rather than statistics were calculated). Unable to calculate RRP diagnosis with this source, we used treatment as a loose proxy for prevalence. Patients were placed into older (age 10-17) and younger (age <10) groups. Trends over time in number and age of patients treated per year and average number of treatments per patient per year were calculated. Rates were generated with population estimates from Vital Statistics data. Trends across pre- and post-vaccination periods were investigated through raw comparisons of these parameters by year and age group.

Results

The average rate of treated RRP per year between 2004-2013 was 0.87/100,000 (range 0.66 to 1.09) for older children and 0.91/100,000 (range 0.60 to 1.10) for younger children. The number of treatments per year in the older group averaged 1.64 (range 1.08-2.14) and in the younger group averaged 2.14 (range 1.93-2.68). No trends in treatment prevalence, age, or treatment frequency in either group were apparent over time.

Conclusion
Despite growing uptakes of HPV vaccination, the rate and frequency of treatment of RRP in NY children have not changed over time. The rarity of RRP and the delay between HPV vaccination and exposure of at-risk infants may explain these results. No central source of US RRP patients exists, however this dataset could be useful for long term monitoring which will be necessary to identify any effect of HPV vaccination on rates of RRP in children.

References


Epidemiology of cervical cancer in a region of southern Algeria

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**Background / Objectives**

Cervical cancer is the second most common cancer and cause of cancer-related death for women worldwide. The early stages of cervical cancer can be free of symptoms, Infection with some types of HPV is the greatest risk factor for cervical cancer. Screening is beneficial in women between 25 and 65 years old. Cytologic test is used in combination with HPV testing.

**Methods**

It’s a prospective analysis of data about cervical cancer from 2016 to 2018 in the province (wilaya) of LAGHOUAT (Algeria). 7 towns was chosen at random. During this two-year study period, 400 cervical screening are required. Actually 200 cervical screening were made. The women age were between 25 and 64 years old.

**Results**

It’s was the first screening for all the 200 women, and 56 percents had only one sexual partner all their life and 12 percent had more than two

the result of cervical intraepithelial neoplasia (CIN) I, II, III was 45, 22, and 15 percent

**Conclusion**

in Algeria an organized screening policy must be set up to reduce the mortality rate from this cancer

**References**


INCREASING TRENDS IN THE INCIDENCE OF POTENTIALLY HUMAN PAPILLOMA VIRUS-ASSOCIATED HEAD NECK CANCER IN ITALY (1988-2012)

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Background / Objectives

High risk alpha human papillomaviruses (HPVs) are recognized to be causally related to a subset of head and neck squamous cell carcinoma (HNSCC) arising from the crypt epithelium of the palatine and lingual tonsils. The aim of this study was to explore the trends in the incidence of HNSCC arising from different anatomical sites potentially related and unrelated to HPV infection among Italian women and men to provide clues on possible growing impact of HPV in the epidemiology of HNSCC in Italy.

Methods

Epidemiological data were retrieved from 10 long-term Cancer Registries of the Network of Italian Cancer Registries (AIRTUM) covering a population of 7.8 million of inhabitants (13% of the whole country) in the period 1988-2012. Trends were described by means of the estimated annual percent change (APC) with appropriate 95% confidence intervals stratified by age, sex, and birth cohort and compared between HPV-related and HPV-unrelated anatomical sites. Only cases with squamous cell histology or morphologic variants of HNSCC were included in the analysis. Cancers arising from lip, nasopharynx, nasal cavity and sinuses were excluded as they are linked to other etiological factors.

Results

A total of 28,883 HNSCCs were included in the analysis. The age-standardized (on European population) annual incidence trends of all sites showed a significant decrease in males (APC: -1.61, 1988-1998; P<0.0001; APC:-3.18, 1998-2012; P<0.0001) and a significant increased in females (APC: +1.41, 1988-2012; P=0.0002). The incidence of cancers arising from head and neck sites strongly related to HPV infection (tonsil and base of tongue/lingual tonsil) increased significantly over the period 1988-2012 (APC: +1.34%; P<0.0001), particularly in females (APC: +2.59%; P=0.0029). Conversely cancers arising from sites poorly related to HPV infection decreased markedly in males and remained relatively stable in females.
Conclusion

The pattern observed suggest a potential increasing impact of HPV infection on the epidemiology of HNSCC in Italy.
P02-07
CERVICAL CANCER IN SITU AMONG WOMEN AGED ABOVE 60 WHO WAS ADEQUATELY Screened At 50S, AND THE POTENTIAL OF PROGRESSING TO INVASIVE CERVICAL CANCER

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Background / Objectives

Nowadays in high-income countries, many women turning age 60 have been adequately screened in their 50s. A previous study found that women who were adequately screened with no abnormality at ages 51-60 do not gain a statistically significant benefit from the extended screening test at ages 61-65 in terms of reducing subsequent invasive cervical cancer. To understand the underlying reason, we performed a population-based cohort study to investigate the cumulative incidence of cervical cancer in situ (CIS) in women aged above 60 who have been adequately screened at age 50s, aiming to predict their likely potential of progressing to invasive cervical cancer.

Methods

Women born between 1919 and 1945 who live in Sweden and had cervical screening records available since age 51 were identified in the Total Population Register. Their screening histories between ages 51-65 were retrieved from the Swedish National Cervical Screening Registry. Women who had at least two separate Pap tests at ages 51-60 without any abnormal finding were included in the analysis. CIS and invasive cervical cancer from age 61 to 80 were retrieved from the National Cancer Register. We estimated the cumulative incidence of CIS up to age 80 among women screened at ages 61-65, and compared to the cumulative incidence of invasive cervical cancer up to age 80 among women unscreened at ages 61-65.

Results

Among 332,746 women who were adequately screened in their 50s, 1.9‰ (95%CI: 1.6‰-2.2‰) of women screened at ages 61-65 were found to have CIS, and 1.6‰ (95%CI: 1.2‰-2.0‰) of women unscreened at ages 61-65 were found to have invasive cervical cancer up to age 80.

Conclusion
Women who were adequately screened in their 50s with no abnormality presented a low risk of CIS after age 60, but these precursors are very likely to progress to invasive cervical cancer since the cumulative incidence of invasive cancer among women unscreened at 61-65 is close to the cumulative incidence of CIS. Therefore, the low effectiveness of cervical screening at ages 61-65 among women being adequately screened previously may due to a low incidence of precursor lesion. However, CIS above age 60 may be risky enough to warrant careful follow-up and treatment. In the scenario that more and more women turning 60 have been adequately screened, screening all of them after age 60 with public resources may not gain satisfactory cost-benefit, given the low incidence of precursor lesions and statistically insignificant effectiveness on cancer reduction. Future studies can endeavor to identify residual risk factors for developing precursor lesions after age 60 despite being adequately screened in the past.
AGE-SPECIFIC ADDITIONAL IMPACT OF A NOVAVALENT HPV VACCINE ON PRECANCEROUS SQUAMOUS CERVICAL LESIONS IN SPAIN


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Background / Objectives

Nonavalent papillomavirus (HPV) vaccine has been licensed in December 2014 and is currently undergoing World Health Organization review for prequalification. Vaccination of girls with HPV bi-quadrivalent vaccines has been widely implemented in Europe and Spain. The bivalent vaccine (Cervarix®, GlaxoSmithKline) targets HPV 16/18, the quadrivalent vaccine (GARDASIL®/Silgard®, Merck&Co) target HPV 6/11/16/18 and the nonavalent vaccine (GARDASIL 9®, Merck&Co) targets HPV 6/11/16/18/31/33/45/52/58. The objective was to describe squamous precancerous cervical lesions potentially prevented by the nonavalent vaccine compared to the bi-quadrivalent vaccines according to age.

Methods

Histologically confirmed cases of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2, n=145) and grade 3-carcinoma in situ (CIN3-CIS, n=244) were studied (2009-2014) in a University Hospital in Spain. High risk HPV genotypes were detected by Linear Array HPV Genotyping test (Roche diagnostics, Mannheim, Germany). The proportion of CIN2-3 lesions potentially prevented by different vaccines was calculated for women 18 to 34, 35-44 and ≥45 years old (age group 1, 2 and 3, respectively). Cervical lesions with coinfection were attributed to the detected genotype belonging to the HPV group most commonly detected in invasive cervical cancer (hierarchical attribution). Ethics Committee of Clinical Investigation of Galicia approved this study. Women signed informed consent. Epidat 3.1 was used for statistical analysis.

Results
Bi-quadrivalent vaccines potentially prevented 59% CIN2 vs. 69% CIN3-CIS ($p<0.001$). Nonavalent vaccine potentially prevented 86% CIN2 and CIN3-CIS. Bi-quadrivalent/nonavalent vaccines potentially prevented 63/87%, 51/91% and 50/75% of CIN2 and 78/90%, 66/86% and 45/76% of CIN3-CIS in age group 1, 2 and 3, respectively. Impact of these vaccines in CIN2-3 tended to decrease with increasing age ($p$-trend $<0.05$). Potential absolute additional impact of nonavalent vaccine was 16%, 26% and 29% of CIN2-3 in age group 1, 2 and 3, respectively, ($p<0.005$).

**Conclusion**

In comparison with bi-tetravalent vaccine, nonavalent vaccine would reduce the gap between CIN2 and CIN3-CIS prevention. Although nonavalent vaccine impact on precancerous lesions decreased as women age increased, significant absolute additional impact was expected in all age groups, especially in women more than 35 years old. Age-specific impact of nonavalent vaccine should be taken into account in cost-effectiveness evaluations and in vaccinated population screening.
HPV VIRAL LOAD CORRELATIONS AMONG YOUNG, RECENTLY-FORMED HETEROSEXUAL COUPLES

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Background / Objectives

High human papillomavirus (HPV) concordance and transmission rates among sexually active couples have been well established. The role of HPV-genotype specific viral load concordance among couples in recently-formed relationships has not been studied well.

Methods

We used data from the prospective HITCH-cohort study. This study included young women (aged 18-24) and their male partners who had recently initiated a sexual relationship in Montreal, Canada, and the couples were followed for up to two years. In the current analyses, we analyzed their web-based questionnaires and genital samples collected at baseline and at four months. Samples were tested for HPV DNA by polymerase chain reaction (PCR) using a Linear Array HPV genotyping assay. In HPV positive samples, viral loads of HPV6, 11, 16, 18, 31, 42 and 51 were quantified using type-specific real-time PCR assays. We assessed the correlation between viral load measurements (number of HPV DNA copies per cell) by calculating Spearman’s rank-based coefficients.

Results

We analyzed 502 couples for HPV DNA, of which 233 couples had at least one partner with a genital sample positive for HPV DNA. Among men and women, 162 and 150 type-specific, persistent HPV infections were detected, respectively. Genital viral loads at baseline were correlated with viral loads at 4 months within individuals with a persistent HPV infection, to a larger extent in men (r=0.413; p<0.001) than in women (r=0.191, p=0.019). Furthermore, in HPV concordant couples, HPV viral loads of sexual partners were correlated with each other at baseline (142 couples, r=0.267, p=0.001). At four months, the magnitude of the viral load correlation decreased in couples that continued having sexual activity with each other (91 HPV-couples, r=0.195, p=0.064). Viral loads of men and women at baseline were not associated with type-specific viral loads of their sexual partner four months later, despite remaining sexually active (r=0.076, p=0.431; r=0.033, p=0.743, respectively).
Conclusion

In individuals with a persistent HPV infection, particularly men, one’s viral load is predictive for the viral load four months later, suggesting limited fluctuations in viral loads over time. HPV viral loads are correlated in young, recently-formed heterosexual couples, but this correlation seems to decrease as the relationship progresses.
THE ONSET OF ORAL SEX, HUMAN PAPILLOMAVIRUS AND OROPHARYNGEAL CANCERS


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Background / Objectives

Human papillomavirus (HPV) is a strong risk factor for a subset of head and neck cancers (HNCs), primarily of the oropharynx (OPC). Sexual behaviours have been suggested as determinants of HPV infections in the oral cavity, but the evidence is inconsistent. Our objectives were to estimate the extent to which oral sex behaviour was associated with an increased risk of OPC, and how much of the association was mediated by oral HPV infection.

Methods

The Canadian site of the HeNCe Life study, an international hospital-based case-control study, recruited 389 incident HNC cases from four hospitals in the Montreal area. A total of 429 controls from outpatient clinics at the same hospitals as the cases were recruited and frequency-matched by age and gender. Life-course oral sex behaviors (including age at first oral sex and time since first oral sex) was collected by semi-structured interviews using a life-grid technique. Oral rinse and oral brush specimens, collected from both cases and controls, were analyzed for alpha HPV genotypes by PCR protocol. Mediation models using logistic regression were used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between life course oral sex behaviors and risk of OPC, adjusting for
Results

A total of 188 OPC cases and 429 controls were included in the analyses. The majority were between 16 and 30 years old the first time having oral sex (63.8% and 55.2% for OPC and controls, respectively). HPV DNA was detected in 63.3% of cases and 14.2% of controls. HPV 16 genotype accounted for 76.5% and 16.4% of HPV positive cases and controls. Age at first oral sex practice was associated with OPC (adjusted OR=2.98; 95%CI 1.37-6.47). When stratified by HPV status, this association decreased (adjusted OR=1.09; 95%CI 0.25-4.71) only in the HPV-positive group. With respect to time since first oral sex, the adjusted ORs were 2.80 (95%CI 1.57-4.97) among all, and 1.04 (95%CI 0.31-3.50) in the HPV-positive group.

Conclusion

Oral sex behaviours were associated with an increased risk of OPC in Canadians, which appears to be mediated by oral HPV infection.
THE PROGNOSTIC ROLE OF DETECTION AND GENOTYPING OF HPV IN PENILE CARCINOMA


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Background / Objectives

A Penile carcinoma (PC) is a rare disease in North America and Europe, a frequent and a serious health problem in developing countries such as Brazil1-3. More than 4600 cases of PC have been registered by the Brazilian National Cancer Institute in 20154. The recurrence or persistence of the infection, including human papillomavirus (HPV), may lead or contribute to the development of penile carcinoma5,6. The prevalence of HPV changes according to the subtypes of squamous cell PC and is 30 to 50% 7. Although it can reach almost 100% in basaloid, in situ and verrucous penile carcinoma8. Objective: Analyze the influence of the presence and detect the type of Human Papillomavirus (HPV) in groin metastasis and specific-cancer survival of patients with penile carcinoma, as well its association to histological variables.

Methods

This retrospective cohort study involved 113 patients with PC treated in the Uro-Oncology service of Hospital Araujo Jorge (HAJ), a unit of the Association Against Cancer in Goias, Brazil (ACCG), from January 2003 to November 2015. This study was approved by the Research Ethics Committee of HAJ. The paraffin blocks containing the cancerous tissue fragments were subjected to extraction of viral DNA using a commercial kit (Promega Corporation, USA), subsequently subjected to polymerase chain reaction testing with short PCR fragment (SPF PCR) primers to detect HPV DNA. HPV genotyping was performed using INNO-LiPA. The HPV 16 and/or 18 presence and its association to other histological profiles were evaluated in penile carcinoma (PC) patients. Uni and multivariate analysis were performed to establish the role of histopathological and HPV on the risk of inguinal metastasis and cancer-specific survival of those patients.

Results
One hundred and thirteen patients were enrolled, forty seven detected with HPV (41.5%). Almost sixty percent of the cases harbored low grade squamous cell carcinoma (SCC). The most prevalent histological subtype was the usual SCC (69.9%), followed by warty SCC (13.3%). The high histological grade (p=0.02) and the presence of HPV18 (p=0.02) were independent prognosticators for specific cancer survival (SCS). Neither the presence of HPV nor the HPV genotype were associated to a higher risk of groin metastasis.

Conclusion

The findings suggest that HPV 18 is an independent factor of poor cancer-specific survival. The overall HPV prevalence (41.5%) and genotype 16 as the most prevalent (51%), followed by HPV18 (23.4%). The importance of HPV in PC is undeniable, although multi-institutional, prospective, collaborative studies concerning this issue are still necessary to better establish its’ prevalence and prognostic impact.

References


P02-12
RECENT INCREASE IN CERVICAL CANCER INCIDENCE IN SWEDEN 2014-2015

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Background / Objectives

From 2014 a clear increase in cervical cancer incidence was observed in Sweden. Using recently available data (June 2017) from the National Cancer Register (NCR) and the National Cancer Screening Register (NKCx) the objective of this study was to investigate this increase by histology, age, and clinical stage at diagnosis.

Methods

Incidence rate ratios (IRR) with 95% confidence intervals (CI) by calendar time were estimated in multivariate Poisson regression models, with interaction terms for histology, age and clinical stage at diagnosis as mediators.

Results

There was an increase in cervical cancer incidence by around 20% (IRR=1.20, CI 1.06-1.36) from 2012 to 2015. This increase was mostly pronounced for adenocarcinoma with a 42% increase in incidence from the period 2012-2013 to 2014-2015 (IRR=1.42, CI 1.18-1.72), while for squamous, adeno-squamous and more rare types of cervical only small, statistically non-significant changes were seen in overall incidence. The increase of adenocarcinoma was mainly concentrated to FIGO stages IA and IB, with a more than 40% increase (IRR=1.45, CI 0.96-2.19 and IRR=1.48 CI 1.15-1.92, respectively). For squamous cancer there was a corresponding increase with around 20% for stage IB cancers only (IRR=1.20, CI 1.01-1.46). For adenocarcinoma the increase in incidence was more pronounced in women below age 50, and for those in stage IB cancers. For squamous cell cancer no statistically significant changes with age could be discerned for the comparison between the two time periods.

Conclusion

The main changes in incidence were seen for early clinical stages, and for adenocarcinoma for women below age 50. Whether these changes in incidence are due to an increase in the underlying risk of cervical cancer or failures of the screening organization still needs to be investigated.
Screening history and the risk of invasive cervical cancer in women aged 66 and older

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Background / Objectives

Cervical cancer is one of the most common cancers in Taiwan. In 1995, the Taiwan government was launched the cervical cancer screening program providing an annual pap smears test of women aged 30 or above. Due to this screening program, the cervical lesions could be diagnosed and treated early. The invasive cervical cancer incidence and mortality rate were decreased after screening program implemented, however, the incidence and mortality rate is still higher in elderly and higher than western countries. The aim of this study was to evaluate the association between pap smear test screening history and the risk of invasive cervical cancer at different age groups.

Methods

In this retrospective cohort study, women aged 36 years or above without cervical cancer history and alive in the end of 2009 were as study subjects. The pap smear screening history was retrospective to 2001-2009 and the incidence of cervical cancer was followed in 2010-2014. Data were obtained from the household registration database, cervical cancer screening registry database, and the cancer registry database. According to the screening history, subjects were classified into 8 groups, including A: regular screening; B: screening attendance in 2004-2006 and 2007-2009; C: screening attendance in 2004-2006, 2007-2009; D: screening attendance in 2007-2009; E: screening attendance in 2001-2004, 2004-2006; F: screening attendance in 2004-2006 sieve; G: screening attendance in 2001-2003; H: never screening. The cumulative incidence of 36-50 years, 51-65 years and over 66 years were estimated by Nelson-Aalen method and hazard ratios were estimated by Cox’s proportional hazards model.

Conclusion

Our study results showed that in women over 66 years old never having pap screening test in 2001-2009 had the highest invasive cervical cancer incidence rate and hazard ratio. Women who had regular screening history, at least once in every three years, has the lowest risk of invasive cancer incidence. Moreover, the longer time interval since last pap test the greater the risk of invasive cervical cancer was increased, especially in women over 66 years. In conclusion, screening history pattern was associated with the risk of invasive cervical cancer in women aged 66 and older. And we need to further consider the feasibility and impact of whether the screening test stops at age 65 in Taiwan.
P02-14
Pilot prevalence of incidence of 12 genotype of high risk HPV and 2 genotype of low risk HPV in Khorasan Razavi State

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Background / Objectives
cervical cancer is the one of common cancer in women. humman papiloma viruis the main factor in etiology. screening of HPV and vaccination can be effective in prevention in this disease.

we intended to determine the prevalence of HPV and genotyping in khorasan razavi.

Methods
In order to detect antibodies against HPV in cervical spicement and DNA of HPV were extracted and tested using PCR to be indentified.

Results
Of 900 case in study, 37 cases(4/1%) werw positive for HPV, and 7 cases(18/9%) were also be positive for low risk HPV, 30 Cases(81/1%) were positive for high risk HPV. also multiple HPV determined in 7 cases. statistical analysis by Chi-2 showed only relation between HPV and passive smoker and duration of using of pil.

Conclusion
Considering the prevalence of HPV in khorasan razavi(4/1%) is lower than report of other countries. but in all of study about efficacy of vaccination, complication and precancerous lesion decreased. we should spread this study in other site of iran that can be determind cost benefit of vaccination.
Presence of HPV in Inverted Papilloma

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Background / Objectives

Inverted papilloma (IP), often referred to as Schneiderian Papilloma, is a locally destructive benign tumour of the sino-nasal mucosa with a tendency for malignant transformation and a high propensity for recurrence. It arises from the transitional epithelium, the Schneiderian membrane. In a Swedish population-based study it was shown to have significantly increased over the last decades. Sinonasal SCC (squamous cell carcinoma) has been shown to be more common among patients with IP than in the general population. The etiology is unknown. Proposed etiological factors are environmental pollutants, organic solvents, smoking and chronic rhino–sinusitis. The possibility of a viral etiology has been put forward where Human Papilloma Virus (HPV) has been the most discussed. Studies on HPV and IP have shown very diverging results. The aim of this study is to analyse the presence of HPV in IP in Stockholm and to investigate if there is any correlation between presence of HPV and recurrence, dysplasia or malignant transformation in IP.

Methods

From the Swedish Cancer Registry we identified all patients diagnosed with IP in Sweden 1960-2010. From the patients in our data set diagnosed from 2001 onwards, diagnosed in the county of Stockholm, we retrieved paraffin embedded blocks with their IP from Biobank Stockholm. After histological re-evaluation of the original diagnosis by a qualified pathologist, and loss of samples due to technical problems, 99 cases out of 126 were included in the study. By analyzing the medical reports we retrieved information about recurrence, dysplasia in the specimens and malignant transformation. The study was approved by the Ethical Committee at the Karolinska Institute, Stockholm, Sweden. The paraffin embedded tumors were cut in 2x15µ sections and DNA was extracted. Detection of HPV was done by PCR using the Magpix(Luminex). The results were confirmed with insitu hybridization.

Results
In all, 13 of the 99 specimens of IP were found to be HPV-positive. 8 were positive for HPV 11, 4 for HPV 6, and 1 for HPV 45. Among the patients with HPV, 4/13 were seen to recur and 4/13 showed dysplasia. This is to be compared with 33/86 and 8/86 respectively in patients with HPV-negative IP. None of the 13 patients with HPV-positive IP developed SCC while two of the HPV-negative did.

**Conclusion**

13% of the IP were HPV positive but only one with a high oncogenic risk HPV. The HPV-positive IP’s were found to have a higher rate of dysplasia compared to the non HPV-positive (30% vs 9%) but no difference was found in their recurrence rate. Patients with HPV-positive IP did not have a higher rate of malignant transformation.
HPV-SPECIFIC B AND T-CELL RESPONSES IN VACCINATED AND NON-VACCINATED YOUNG WOMEN.

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Background / Objectives

A primary HPV infection is cleared naturally in about 90% of the cases, suggesting that the immune system plays an important role in the protection against HPV-associated diseases, like cervical cancer. The HPV vaccines induce high HPV-specific antibody levels and memory B cell responses. Moreover, T-cells are known to be of importance in cell-mediated immunity. However, HPV-vaccine induced cell-mediated responses are not well understood after the HPV vaccination. We aim to investigate the cellular immune responses comparing non-infected, transient- and persistent infected young women.

Methods

In a longitudinal follow-up study, vaccinated and non-vaccinated young women were followed for 7 years post vaccination. From a part of all individuals peripheral blood monocytes (PBMC) were isolated (n=100 per year). Memory B-cell responses will be measured by HPV-serotype specific ELISpot assay using virus like particles, assembled from the major capsid protein L1 for HPV-16, HPV-18, HPV-31 and HPV-45. T-cell responses will be determined by cytokine production of stimulated PBMCs with HPV-specific peptides pools.

Results

Memory B-cell responses and T-cell responses of participants with persistent HPV infection will be compared with those of participants who had no HPV infection or cleared their HPV infection. These immune responses will be further evaluated in the vaccinated and non-vaccinated groups. HPV-specific frequencies of memory B-cells will be determined and T-cell responses will be expressed in numbers of HPV-specific cytokine producing T-cells.

Conclusion
Obtained results will provide more insight and understanding in the immune mechanism of HPV-specific cellular responses.
HR-HPV L1, E1, E2, E6, E7 SEROPOSITIVITY DOES NOT PREDICT ANAL HSIL AMONG HIV-POSITIVE MEN WHO HAVE SEX WITH MEN


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Background / Objectives

High-risk HPV L1 and E6/E7 seropositivity is prospectively associated with anal cancer. We studied L1, E1, E2, E6, E7 seropositivity of high-risk (hr) HPV types as potential predictors of anal high-grade squamous intraepithelial lesions (HSIL) among HIV-positive men who have sex with men (MSM).

Methods

HIV-positive participants of the longitudinal HIV&HPV in MSM (H2M) study who had at least two visits and a high-resolution anoscopy (HRA) after the last H2M visit were included in this analysis. Sera were collected in 2010-2013. Serum antibodies to E6, E7, and L1 proteins of 7 hr-HPV types (16, 18, 31, 33, 45, 52, 58), and serum antibodies to E1 and E2 of HPV16 and HPV18 were analyzed by multiplex serology. Seropositivity was defined as 3 out of 4 positive among E1/E2/E6/E7 for HPV16 and HPV18; and both E6 and E7 positive for each non-HPV16/18 type. Univariable and multivariable logistic regression was used to assess whether hr-HPV seropositivity was predictive of HSIL.

Results

Among 193 MSM (median age 50 years [IQR]: 45-56) 60 (31%) were diagnosed with histologically proven anal HSIL: 25 (13%) AIN2 and 35 (18%) AIN3. The median nadir CD4+ was 235 cells/µl (IQR: 150-315 cells/µl), and 94% had an undetectable HIV viral load at time of HRA. Seropositivity for E1, E2, E6, E7 of HPV16 was 7%, 4%, 4%, and 5%, respectively. In total, 0 (0%) were HPV16 three out of four positive for E1/E2/E6/E7, and 0 (0%) HPV18 three out of four positive for E1/E2/E6/E7. E6 and E7 seropositivity for each of the non-HPV16/18 hr-HPV types was 0% (n=0).
Type-specific seropositivity as defined above was not associated with HSIL diagnoses.

Conclusion

No association between type-specific hr-HPV seropositivity and anal HSIL was found among HIV-positive MSM. Our analysis shows that (type-specific) hr-HPV seropositivity cannot be used as predictor of HSIL in HIV-positive MSM.
SEROTYPE AND GENETIC DIVERSITY OF HUMAN PAPILLOMAVIRUS 58 IN ITALIAN WOMEN WITH LOW-GRADE CYTOLOGY

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Background / Objectives

Persistent infection with high-risk HPV genotypes is highly associated with the development of cervical cancer. HPV58 is a member of the HPV16-related alpha9 family and accounts for approximately 2% of all cervical cancer cases worldwide. Genetic variants of HPV58 have been classified into four major lineages, A, B, C and D and seven sub-lineages A1, A2, A3, B1, B2, D1 and D2, the distribution of which varies by geographical region. Lineage A predominates in all regions except in Africa, where lineages A and C are found in comparable proportions.

The aim of this study was to evaluate the potential influence of common HPV58 L1 and L2 polymorphisms on capsid protein recognition by antibodies elicited by natural infection.

Methods

HPV58 L1L2 pseudoviruses (PsVs) representing the eight major L1 and L2 variant lineages (A1, A2, A3, B1, B2, C, D1 and D2) were generated. Paired serum and DNA samples collected from women following a diagnosis of ASCUS or LSIL were tested for the presence of neutralizing antibodies against HPV58. HPV58 DNA positive samples from patients with evidence of seroconversion against HPV58 were subjected to fragment sequencing to identify their lineage variant status.

Conclusion

Among the 216 serum samples tested, 31 were seropositive against all HPV58 variants with the exception of the C variant. One serum sample was positive for HPV58 C variant, but did not recognise any other variants. Out of 32 seroconverted individuals, 21 (65%) were also DNA positive against HPV58 of which 19 were infected with HPV58 A2, one with HPV58 C, and one with HPV58 B2. We are currently mapping target specificity of these antibodies by construction of inter-lineage loop swap PsV. These data demonstrate that naturally occurring polymorphisms in the HPV58 capsid proteins affect recognition by antibodies elicited during natural infection and suggest the existence of lineage-level serotypes.
Human papillomavirus prevalence and genotype distribution in urine samples from vaccinated as compared to non-vaccinated females in Norway

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Background / Objectives

Quadrivalent HPV vaccine was included in the Norwegian childhood immunisation programme in September 2009 to girls in 7th grade. At present, 88% of all eligible girls have received at least one dose, and 86% all three vaccine doses. Since November 2016, catch up vaccination for girls up to 26 years is currently offered in a 2-year programme. In the national HPV-surveillance programme, HPV-testing in urine is used to monitor the impact of HPV vaccination on HPV prevalence and type distribution in pre-screening age. Two HPV prevalence base-line studies have previously been performed in non-vaccinated cohorts at age 17. The HPV-prevalence was 19.9% (girls born 1994) and 15.5% (girls born 1996), respectively. In this study, we include also the first vaccinated cohort (born 1997). We present preliminary results of the impact of HPV-vaccination on HPV-prevalence and genotype distribution in 17-year old girls. The study was conducted prior to the start of the catch-up programme.

Methods

Two birth cohorts of unvaccinated 17 year old girls (n=56,000) and one birth cohort of vaccinated 17 year old girls (n=30,000) were invited by mail to participate in the study. Sampling materials were sent to all girls who signed the informed consent form. The presence of HPV was investigated by using a modified GP5+/6+ PCR protocol, followed by hybridization of type-specific oligonucleotide probes coupled to fluorescence labeled polystyrene beads (Luminex suspension array technology), detecting and genotyping 37 HPV types (WHO validated protocol). Sample adequacy was evaluated through a beta-globin PCR. Individual vaccination records were retrieved from the Norwegian immunisation register, and HPV-prevalence in vaccinated and unvaccinated girls were compared.

Results

Preliminary results show a significant reduction in overall HPV prevalence in vaccinated as compared to unvaccinated 17-year old girls. The prevalence of high-risk vaccine types 16 and 18 were dramatically reduced, and also for non-vaccine
types a reduced prevalence was observed. Analyses are ongoing and detailed results will be presented in the poster.

Conclusion

In this large, population based study, a high effectiveness of the HPV-vaccination programme for 12 year old girls was demonstrated, and HPV-testing in urine samples was found to be easy and highly feasible for vaccine surveillance in adolescent girls. Except from HPV type 11, quadrivalent vaccine targeted HPV types (HPV 6, 11, 16 and 18) were among the most prevalent types in the unvaccinated cohorts. The prevalence of vaccine types was greatly reduced in vaccinated girls at age 17, and clear evidence of cross-protection of non-vaccine types was observed.

References


Background / Objectives

Background

In 2016, the Korean government launched a national HPV vaccination programme for 12 year old girls (bivalent and quadrivalent HPV vaccines) and approved the use of 9-valent HPV vaccine. This is expected to have a significant impact on HPV-related disease burden in Korea. The aim of this review is to examine the current burden of HPV-related cancers and disease and to estimate the relative contribution of the nine vaccine types (HPVs 16/18/31/33/45/52/58/6/11).

Methods

Methods

A comprehensive search of peer-reviewed biomedical literature was conducted to assess the burden of HPV disease in Korea by using MEDLINE, Asian Pacific Journal of Cancer Prevention, KoreaMed Synapse and Google Scholar until August 2016.

To assess the potential impact of the 9vHPV vaccine in HPV-related lesions, we used data from an international project on HPV-related lesions designed and coordinated by the Catalan Institute of Oncology (ICO) (Barcelona-Spain). Consecutive histologically confirmed paraffin-embedded cases of HPV-related anogenital cancers (cervix, vulva, vagina, anus and penis) were obtained from Korean hospital pathology archives. Cancer sites with a limited number of cases were supplemented with cases from the Asian region. HPV DNA-detection and typing was performed by using SPF10-DEIA-LiPA25 system and relative contribution was expressed as the proportion of type-specific cases among HPV positive samples.
Results

Despite a downtrend in cervical cancer rates in recent years, Korean rates still remain high in comparison to other developed countries (age-standardized rate in 2012: 9.5 cases per 100,000 women). HPV-related anogenital cancers other than cervix remain rare. Preliminary results show that the combined relative contribution of the nine HPV vaccine types was 91.3% (95% CI: 89.9-92.6) in cervical cancer, 73.6% (95% CI: 51.6-89.8) in vaginal cancer, 83.3% (95% CI: 70.7-92.1) in vulvar cancer, 88.9% (95% CI: 51.6-99.7) in penile cancer and 91.3% (95% CI: 72.0-98.9) in female anal, and 88.2% (95% CI: 63.6-98.5) in male anal cancer. The most frequently detected types in cervical cancer are HPV 16 (65%), HPV 18 (9%), HPV 33 (5%), followed by HPVs 58 (4%) and 31 (4%). HPV16 was the most frequent type in all lesions.

Conclusion

HPV-related disease burden in Korea is significant. Results suggest that the HPV types in the 9vHPV vaccine contribute to more than 90% of HPV positive female cervical and anogenital lesions. Consequently, the introduction of the 9vHPV vaccine could have a significant impact on the prevention of HPV-related cancer and disease in Korea.
SAFETY AND EFFICACY OF A QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE AGAINST PERSISTENT INFECTION AND GENITAL DISEASES IN CHINESE WOMEN DURING A 78-MONTH FOLLOW-UP

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Background / Objectives

Human Papillomavirus (HPV) infection causes significant disease burden in China. Here we report a randomized, double-blind, placebo-controlled multicenter trial conducted in Chinese healthy women to assess the safety and efficacy of a quadrivalent HPV (types 6, 11, 16, 18) L1 virus-like–particle vaccine (Gardasil®) against persistent infection and genital diseases.

Methods

3006 participants aged 20 to 45 years were enrolled and randomized (1:1) to receive HPV vaccine or placebo at Day 1, Month 2 and 6. The efficacy was followed up till Month 78. The primary efficacy endpoint was HPV 16/18-related cervical intraepithelial neoplasia grade 2 or 3 (CIN 2/3), adenocarcinoma in situ (AIS) or cervical cancer. Other efficacy endpoints included HPV 6/11/16/18-related: 1) CIN of any grade, AIS or cervical cancer (CIN plus); 2) 12-month persistent infection (PI); 3) 12-month PI, CIN plus or external genital lesions (EGLs, including genital warts, vulvar or vaginal intraepithelial neoplasia, vulvar or vaginal cancer); 4) EGLs. The efficacy analyses were done on the type-specific per-protocol efficacy (PPE) population who received all the 3 doses and were naïve to the relevant HPV types through 1 month after the third dose. Injection-site and systemic adverse events (AEs) were recorded within 15 days after each dosing. Serious AEs (SAEs) in the participants and their infants/fetuses, and pregnancy outcomes were collected throughout the study. (ClinicalTrials.gov registry: NCT00834106)

Results

0 and 7 cases of HPV 16/18-related CIN2/3, AIS or cervical cancer were observed among 1,265 and 1,237 participants in the vaccine and placebo groups, respectively, translating into an efficacy of 100% (95%CI: 32.3, 100). The efficacies against HPV 6/11/16/18-related genital diseases or infection were: 1) 100% (95%CI: 70.9, 100) for CIN plus; 2) 91.0% (95%CI: 77.7, 97.2) for 12-month PI; 3) 91.8% (95%CI: 79.8,
97.4) for 12-month PI, CIN plus or EGLs. No EGLs case was observed. 926 (61.8%) and 856 (57.1%) participants reported AEs in the vaccine and placebo groups, respectively. Injection-site AEs were more frequent in the vaccine group (37.6% vs. 27.8%, p<0.001). Systemic AEs incidences were similar (51.4% vs. 50.1%). 38 (2.5%) and 43 (2.9%) participants reported SAEs in the vaccine and placebo groups, respectively. Incidences of congenital anomaly in infants and aborted fetuses were 2.3% (11/488) in vaccine group and 1.4% (6/444) in placebo group (p=0.3371).

Conclusion

The quadrivalent HPV vaccine demonstrated good safety profile and high efficacy against persistent infection, any-grade and high-grade cervical precancerous lesions in Chinese healthy adult women.
P05-04
POTENTIAL OF HPV VACCINATION IN CANCER CONTROL

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Background / Objectives

Human papillomavirus (HPV) is involved in the pathogenesis of anogenital cancers and oropharyngeal cancer (OPC) in both women and men. HPV-vaccination is approved for prophylactic use in both genders. HPV-related cancer is increasing in men, but in most countries, including Denmark, HPV-vaccination is recommended for girls only. The objective of this study was to estimate the burden of cancers caused by HPV in women and men and the potential of HPV-vaccination in cancer control.

Methods

We retrieved data on the prevalence of HPV and the genotype distribution in HPV-related cancer types from the existing literature. Data on cancer incidences as well as frequency of procedures related to cervical screening was searched from the NORDCAN database and Danish National Health Registers.

Results

Every year, 376 Danish women are diagnosed with cervical cancer and 99 women die from the disease. The incidence of cervical cancer has declined from 44.4 per 100,000 before cervical screening was introduced in Denmark in the 1960’s to 14.5 per 100,000 today. In Denmark, 700 women and 384 men are diagnosed with an HPV-related cancer each year. The annual burden of HPV-caused cancer was estimated to 548 new cases in women compared to 234 cases in men. The Danish cervical screening program is estimated to prevent 800-1300 cervical cancer cases each year. If these preventable cases are included in the estimate, the burden of HPV-caused cancer is 6-8 times higher in women than in men.

Conclusion

The burden of cancer caused by HPV and the preventive potential of HPV-vaccination is twice as high in women compared to men. However, what we see today is only the tip of the iceberg. When the cervical cancer cases prevented by screening are taken into account, the burden of HPV-caused cancer and the preventive potential of HPV-vaccination is considerably higher in women than in men. In Denmark, the coverage of HPV-vaccination has declined dramatically due to public concern about possible side-effects. Gender-neutral HPV-vaccination potentially would benefit girls more than boys, and the coverage in boys would have to be very high if coverage in girls is low.
P05-05
HPV vaccination and risk of chronic fatigue syndrome/myalgic encephalomyelitis: A nationwide register-based study from Norway

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Background / Objectives

Vaccination has been suggested in the aetiology of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). However, for the vaccines studied, including the bivalent HPV vaccine, no associations have been found [1, 6-9]. The risk of developing CFS/ME after vaccination with the quadrivalent HPV-vaccine has not been studied. Recently, an increased risk of two syndromes with symptoms that partly overlap with CFS/ME (postural orthostatic tachycardia syndrome and complex regional pain syndrome) after HPV vaccination has been suspected [10-12]. From 2009, quadrivalent HPV vaccine has been offered to 12 year old girls in the Norwegian Childhood Immunisation Programme. We studied whether HPV vaccination was associated with risk of CFS/ME and assessed medical history in relation to both risk of CFS/ME and HPV vaccine uptake. Uptake of at least one dose increased from 70% to 88 % in the study period, 2009-2014.

Methods

We linked individual data from national registries, including the population registry, the patient registry and the immunisation registry using the unique personal identification number.

Yearly incidence rates of CFS/ME for 2009–2014 were calculated among all boys and girls, aged 10–17 living in Norway during the period, n=824,133.

Girls born 1997–2002 were eligible for HPV vaccination and included in analyses of the interplay between vaccination, medical history and CFS/ME, n=176,453.

We calculated hazard ratios (HRs) of CFS/ME using Cox regression. Risk differences (RDs) of vaccine uptake were calculated with binomial regression.

Results
Although the incidence of CFS/ME was higher among girls than boys, we observed a similar yearly increase in incidence rate of CFS/ME among girls and boys.

Among girls eligible for HPV vaccination, the risk of CFS/ME increased with increasing number of previous hospital visits, HR=5.23 (95% CI 3.66–7.49) for seven or more visits as compared to no visits. Having seven or more hospital visits was associated with a lower HPV vaccine uptake, RD=−5.5% (95% CI -6.7%—4.2%).

We observed no association between HPV vaccination and risk of CFS/ME, HR=0.86 (95% CI 0.69–1.08) for the entire follow-up period and 0.96 (95% CI 0.64–1.43) for the first two years after vaccination.

**Conclusion**

We observed an increase in CFS/ME incidence during 2009–2014. The increase was similar in girls and boys. Further, an association between medical history and risk of CFS/ME was observed, and the risk increased with increasing number of hospital visits.

Analysing individual data, no indication of increased risk of CFS/ME following HPV vaccination was found among 176,453 girls offered HPV vaccine through the national immunisation programme in Norway 2009-2014.

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HPV vaccination of adolescent girls is not associated with sexual activity initiation and risky sexual behaviours

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Background / Objectives

Some fear that HPV vaccination may lead to an increase in unfavourable sexual health outcomes, based on the theory of risk compensation suggesting that the relative assurance of the protection from vaccination could be associated with an increase in risky sexual behaviours. Our study aimed to test whether receiving an additional dose of Q-HPV vaccine between the ages of 13 and 15, five years after the initial dose was received, would lead to more sexual activity and more risky sexual activity over a year, among teenage girls vaccinated in Quebec, Canada.

Methods

We analyzed data collected as part of an ongoing randomized trial, ICI-VPH, investigating the role of a booster dose of HPV vaccine. All participants received 2 doses of Q-HPV vaccine in fourth grade. The intervention group received an additional dose 60 months after their first one; the control group did not receive a vaccine booster dose. Girls included in the present analysis were those who had no sexual experience at the time of the randomization and who responded to the follow-up questionnaire one year later. The main outcome was the occurrence of the first sexual experience in the year following randomization. Secondary outcomes included: lifetime number of sexual partners, condom use, STIs and pregnancy.

Results

Of 1581 girls, 798 (50.5%) received an additional Q-HPV vaccine dose and 783 (49.5%) did not. At the time of randomization, groups showed similar characteristics: the mean age was 14.8 years, 70.5% self-identified as French Canadian only, 91.3% were born in Canada, 12.0% were using hormonal contraception and 4.5% were smokers. In the year following randomization, similar proportions of participants initiated sexual activity (17.2% vs 19.9%; p-value 0.26); initiated intercourse (14.9% vs 16.4%; p-value 0.24); and used condoms (67.5% versus 63.4%; p-value 0.57). Only 2 participants reported an STI (one in each study group), and one reported a pregnancy (in the control group). In multivariate analysis, identifying as French Canadian only (OR 1.5; 95% CI: 1.1-2.0), tobacco smoking (OR 3.0; 95% CI: 1.8-5.1) and hormonal contraception use (OR 2.4; 95% CI: 1.7-3.4) were associated with sexual activity initiation.
Conclusion

We did not observe an increase in sexual activity, risky sexual behaviours or unfavourable sexual health outcomes in adolescent girls who received an additional dose of HPV vaccine between 13 and 15 years of age.
P05-07
CROSS-PROTECTIVE EFFECTIVENESS OF AS04-HPV-16/18 VACCINATION IN REDUCING CERVICAL HPV INFECTIONS IN ADOLESCENT GIRLS – RESULTS FROM A COMMUNITY-RANDOMIZED TRIAL

M. Lehtinen 1, D. Apter 2, T. Eriksson 1, K. Natunen 1, J. Paavonen 3, S. Damaso 4, D. Bi 4, F. Struyf* 4

1University of Tampere, Tampere (Finland), 2VL-Medi Clinical Research Center, Helsinki (Finland), 3Helsinki University Hospital, Department of Obstetrics and Gynecology and University of Helsinki, Helsinki (Finland), 4GSK, Wavre (Belgium)

Background / Objectives

Aside of the high protection against the most prevalent carcinogenic HPV types (16/18) provided by the AS04-adjuvanted HPV-16/18 vaccine (AS04-HPV-16/18v), large efficacy trials have evidenced its protective effect against some non-vaccine oncogenic types. We present results from a post-hoc analysis on cross-protective vaccine effectiveness (VE) against non-vaccine HPV type cervical infections in adolescent girls from a large phase III/IV, community-randomized, controlled study (NCT00534638).

Methods

From 2007 to 2010, 22,444 girls and 11,968 boys from Finland born 1992-95 (aged 12-15 years) were allocated to 3 arms. Around ninety percent of vaccinated girls and boys in arm A (8,235/9,203) and of vaccinated girls in arm B (6,601/7,367) received AS04-HPV-16/18v. Other vaccinated subjects in arms A and B (6,614) and all in arm C (10,724) received hepatitis B virus vaccine.

HPV DNA prevalence of 14 high-risk and 11 low-risk types in cervical samples collected from female subjects when they were 18.5-19 years old was determined by SPF-10 line probe assay (LiPA) and Multiplex Type-specific PCR.

VE was calculated as a relative reduction of HPV prevalence by type in cervical samples among HPV-vaccinated girls from pooled arms A & B compared with non HPV-vaccinated girls from arm C (control arm). The analysis was performed on the total enrolled cohort (TEC), overall and by birth cohort (92-93 and 94-95), accounting for the differences in average age at vaccination (14-15 and 13-14 years old) and time to follow-up (3-4 and 5-6 years).

Results

VE are presented for HPV-31, 33, 35 and 45 (no significant changes were shown for other types).
<table>
<thead>
<tr>
<th>HPV type</th>
<th>TEC</th>
<th>Arm</th>
<th>N</th>
<th>n</th>
<th>VE (%)</th>
<th>95% CI (LL-UL)</th>
<th>p-value</th>
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<td>Overall</td>
<td>A&amp;B</td>
<td>5,853</td>
<td>51</td>
<td>81.1</td>
<td>70.7–87.8</td>
<td>&lt;0.001</td>
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<td>92-93</td>
<td>A&amp;B</td>
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<td>79.3</td>
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<tr>
<td></td>
<td>94-95</td>
<td>A&amp;B</td>
<td>2,778</td>
<td>25</td>
<td>80.3</td>
<td>68.1–87.8</td>
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<tr>
<td>33</td>
<td>Overall</td>
<td>A&amp;B</td>
<td>5,853</td>
<td>121</td>
<td>47.4</td>
<td>29.2–60.9</td>
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<td>A&amp;B</td>
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<td>35</td>
<td>Overall</td>
<td>A&amp;B</td>
<td>5,853</td>
<td>43</td>
<td>54.2</td>
<td>30.0–70.0</td>
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<td>74.6</td>
<td>55.5–85.5</td>
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<td>A&amp;B</td>
<td>2,778</td>
<td>9</td>
<td>75.9</td>
<td>47.1–89.0</td>
<td>&lt;0.001</td>
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<td>C</td>
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CI: confidence interval; LL-UL: lower and upper limits; N: number of subjects; n: number of positive samples; TEC: total enrolled cohort; VE: vaccine effectiveness

All analyses are exploratory. CI and p-value are based on Mantel-Haenszel adjusted for clustering and stratified by the historical HPV-16/18 seroprevalence used in the randomization.

Conclusion

Cross-protective effectiveness of AS04-HPV-16/18v against non-vaccine HPV type (31/33/35/45) cervical infections was observed in adolescent girls 3-6 years post vaccination. Protection appeared higher in younger birth cohorts.

References
Funding: GlaxoSmithKline Biologicals SA

*On behalf of the HPV-040 study group
EFFICACY OF THE QUADRIVALENT HPV VACCINE IN CERVICAL CANCER PREVENTION STRATEGY IN THE GAMBIA.

Persistent infection with high risk Human Papillomavirus (HR HPV) genotype causes 80% of cervical cancers. HR HPV 16 and 18 are responsible for 70% of cervical cancers, worldwide. Three prophylactic HPV vaccines have been developed to prevent HPV infections. In the Gambia, cervical cancer is the most frequent diagnosed cancer representing approximately 30% of all female cancers. The quadrivalent HPV vaccine, which targets genotypes 16, 18, 6 and 11 was recently piloted in the West Coast Region where majority of cervical cancer cases were reported. In order to evaluate the potential efficacy of the quadrivalent vaccine, this study assessed regional genotype distribution to ensure the HPV vaccine prevention strategy would be effective.

Methods

232 endocervical samples were collected from women age 20 - 49 years old residing in Banjul and West Coast Region. DNA was isolated using the QIAamp DNA Mini Kit (Qiagen). HPV detection was carried out by PCR amplification using primer sets PGMY09/11, which target the (L1) Major capsid gene of the virus. Genotyping was performed by Sanger sequencing technique.

Results

Eight different HR HPV genotypes were identified. HPV 52 (28.6%) was the most prevalent genotype, followed by 58 and 51 (both 14.2%). HPV 16 (7.1%) was the seventh most common genotype identified and HPV 18 was not detected. HR HPV distribution was higher in the 26-30 age group. HPV 61 was the most common low risk genotype isolated. Sequence analysis showed all HR genotypes detected were not homologous to African isolates but isolates originated from America, Europe and Asia.

Conclusion
The success of a cervical cancer vaccine prevention strategy should consider the dominant circulating HR HPV type. In the Gambia, the vaccine currently available may be of limited use.
MOTHER TO INFANT TRANSFER OF ANTI HPV 6 AND 11 ANTIBODIES UPON IMMUNIZATION WITH THE 9VHPV VACCINE

A. Joshi, A. Luxembourg

Merck & Co., Inc., Kenilworth, NJ (United States of America)

Background / Objectives

HPV types 6/11 can cause recurrent respiratory papillomatosis (RRP), a rare disease with likely mother to child transmission. This exploratory analysis was conducted to characterize the level of HPV types 6/11 antibodies in peripartum maternal blood and in cord blood of infants born to women who received 9-valent HPV (types 6/11/16/18/31/33/45/52/58) (9vHPV) vaccine in the pivotal efficacy study of the 9vHPV vaccine (V503-001, NCT 00543543). Immunization with the 9vHPV vaccine has been shown to elicit marked antibody responses to all 9 vaccine types; however limited data exist on the maternal transfer of anti HPV antibodies.

Methods

The overall efficacy study enrolled over 14,000 subjects who were randomized to 9vHPV vaccine or quadrivalent HPV (qHPV) vaccine. Participation in the sub study to assess mother to infant HPV antibody transfer was voluntary. The analysis included all mothers and infants for whom valid results for maternal blood and infant cord blood samples were available at the time of delivery. A total of 20 mother-infant pairs for HPV 6 (n=9; 9vHPV group and n=11; qHPV group) and 21 mother-infant pairs for HPV 11 (n=9; 9vHPV group; n=12; qHPV group) were analyzed. Geometric mean titers (GMTs) and seropositivity rates of anti-HPV 6/11 neutralizing antibodies in the mother-infant pair samples were assessed using competitive Luminex immunoassay.

Results

All mothers and all infants were seropositive for HPV 6 and HPV 11. Anti-HPV 6/11 GMTs in peripartum maternal blood and infant cord blood were highly correlated. The GMT ratios of peripartum maternal blood vs. those in cord blood were 1.23 (95% C.I.; 0.43, 3.49) for HPV 6 and 1.29 (95% C.I.; 0.54, 3.07) for HPV 11 in the 9v HPV group and 1.33 (95% C.I.; 0.41, 4.29) for HPV 6 and 1.19 (95% C.I.; 0.45, 3.13) for HPV 11 in the qHPV group, respectively.

Conclusion

These results indicate that antibodies induced by the 9vHPV vaccine cross the placenta and could potentially protect newborns against acquisition of vaccine type HPV related disease, such as RRP. These results mirror similar observations previously made with qHPV vaccine.
Long Term Immunogenicity, Efficacy and Safety of 9-valent HPV vaccine in Preadolescents and Adolescents.

S.E. Olsson ¹, A. Luxembourg ²

¹Karolinska Institute at Danderyd Hospital, Stockholm, Sweden (Sweden), ²Merck & Co., Inc., Kenilworth, NJ, USA (United States of America)

Background / Objectives

The efficacy of the 9vHPV vaccine, developed to prevent HPV infection and disease caused by HPV6/11/16/18/31/33/45/52/58, was demonstrated in a Phase III study (Study 001) in young women (aged 16 to 26 years). In another Phase III study (Study 002), the efficacy results were bridged to girls and boys (aged 9-15 years) based on the demonstration of non-inferior HPV antibody responses compared to young women. Study 002 was extended to evaluate vaccine immunogenicity, efficacy and safety over 10 years. An interim analysis of immunogenicity of Study 002 up to 3 years and plans for longer term immunogenicity and effectiveness follow up will be presented.

Methods

Young women aged 16-26 years (Study 001) and girls and boys aged 9-15 years (Study 002) received 3 doses of 9vHPV vaccine at day1, month 2 and month 6. Serology was assessed at month 7, 12, 24, 36, using HPV-9 cLIA. Vaccine immunogenicity is estimated in the per-protocol population by assessing geometric mean titers (GMTs) and seropositivity rates to each vaccine type HPV. Non-overlapping 95% confidence intervals were used as indicators of differences of immune response.

Results

In Study 002, seropositivity rates to each of the 9 HPV types in girls and boys ranged from 99.9% to 100% at month 7 and from 93.8% to 99.7% at month 36. GMTs peaked at month 7, and decreased thereafter to plateau between month 24 and month 36. An analysis by age strata (9-12 years and 13-15 years at enrollment) showed that the month 36 seropositivity rates ranged from 96.5% to 99.7% in the younger group and 87.2% to 99.7% in the older group. This difference in GMTs by age strata was statistically significant in girls at all-time points; differences in boys were smaller and were not statistically significant.
Efficacy of the 9vHPV vaccine was established through 6 years of follow-up (median 4 years) in young women in Study 001. A cross-study comparison showed that GMTs in girls and boys from Study 002 were higher than GMTs in young women from Study 001 at month 7, and remained higher throughout the study. Based on these results, efficacy in girls and boys through month 36 is inferred. Study 002 was extended to continue assessment of antibody persistence and initiate assessment of effectiveness (through 10 years post vaccination).

Conclusion

Administration of the 9vHPV vaccine in girls and boys aged 9-15 years resulted in HPV antibody responses that persisted through 3 years. HPV antibody responses remained higher in girls and boys than in young women (the population used to establish 9vHPV vaccine efficacy) for this entire study period. Longer term assessment of immunogenicity and effectiveness is ongoing.
WOULD THE RESTORATION OF THE VAGINAL MICROBIOTA HELP THE HPV REGRESSION?

L. Serrano, J. Cortés, A.C. López, S. González, S. Palacios, D. Dexeus, C. Centeno

Centro Médico Gabinete Velázquez (Madrid) (Spain), Private Practice (Palma de Mallorca) (Spain), Hospital Quironsalud (Málaga) (Spain), Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), Women’s Health Institute (Barcelona) (Spain), Clínica Diatros (Barcelona) (Spain)

Background / Objectives

There is increased evidence of higher diversity of the vaginal microbiota of HPV-positive Vs HPV-negative women. Bacterial species among HPV infected patients are possible cytokine profile modifying agents (Th1 to Th2), causing local immunosuppression resulting in HPV persistency. Thus, re-balance or normalization of the microbiota, may help to produce a more hostile microenvironment for HPV, thereby making easier its clearance.

Methods

Review of 3 prospective studies:

- Exploratory, non-comparative, prospective, real life study conducted on healthy women aged 18 - 45 years, once daily application of Papilocare® for 12 consecutive days to measure changes in vaginal microbiota.

- Prospective, non-controlled observational study including 21 sexually active positive-HPV women aged > 25y with negative pap and no colposcopy cervical lesions. PapilocareR once daily for 21 consecutive days to evaluate changes in vaginal microbiota by pyrosequencing.

- Randomized, open, parallel group, controlled clinical trial to evaluate the efficacy of Papilocare® to both normalize cytology and clear HPV, in HPV-positive women with ASCUS or LSIL alterations and consistent colposcopy image.

Results

- First study showed a trend of improvement (21.2% final vs baseline) of vaginal microbiota.

- Second study showed a significant improvement in the cervix mucosa epithelialization vs baseline. Evaluation of changes in vaginal microbiota by pyrosequencing are under analysis and will be disclosed during Congress.

Third study interim analysis:

- At 3 months, 69.2% of patients using Papilocare® (n=26) negativized pap and...
colposcopy vs. 33.3% in control group (n=15) (p=0.048; Fisher test). This difference is even more evident in high risk genotype population: 67% vs 20% for PapilocareR (n=18) and control group (n=10), respectively (p=0.046; Fisher test)

- At 6 months, a positive trend of Papilocare® vs control in normalizing Pap and colposcopy in high risk genotype population: 73% vs 40% in PapilocareR (n=11) and control groups (n=5), respectively (p=ns)

- At 6 months, a positive trend to clear HPV in Papilocare® vs control group: 56% vs 30% of patients cleared HPV, respectively (p=ns). This positive trend was even more evident in high risk genotype population: 50% of patients in PapilocareR group (n=12) showed HPV cleared vs 17% (n=6) in control group (p=ns)

Conclusion

Papilocare® shows a positive outcome on vaginal microbiota which may enhance local immunity and might explain that Papilocare® shows a significant normalization Pap at 3 months vs control and a positive trend in both HPV clearance at 6 months with higher differences in high risk HPV patients. These findings need to be confirmed upon study completion
EFFICACY OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL TO REPAIR CERVICAL MUCOSA WITH HPV LESIONS. INTERIM ANALYSIS RESULTS

L. Serrano 1, J. Cortés 2, A.C. López 3, S. González 4, S. Palacios 4, D. Dexeus 5, C. Centeno 6

1Centro Médico Gabinete Velázquez (Madrid) (Spain), 2Private Practice (Palma de Mallorca) (Spain), 3Hospital Quironsalud (Málaga) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women´s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain)

Background / Objectives

To evaluate the efficacy of Papilocare®-a Coriolus versicolor-based vaginal gel- to repair cervical mucosa in women with HPV-related pap alterations and consistent colposcopy image.

Methods

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US or LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment. Preliminary analysis of percentage of patients with normal pap and concordant colposcopy image at 3 months in both total and high risk genotype virus population are presented. Citologies evaluation has been centrally-conducted in IECM laboratory (Lugo, Spain). Papilocare® arms (A+B) were combined for this analysis.

Results

Data from 41 patients at 3 months are available. 69.2% of patients using Papilocare® (n=26) had negative pap and colposcopy vs. 33.3% in control group (n=15) (p=0.048; Fisher test).

High risk genotypes virus were detected in 28 patients. At 3 months, normal pap and concordant colposcopy image was observed in 67% of patients using Papilocare® (n=18) vs 20% of patients in control group (n=10) (p=0.046; Fisher test).

Conclusion

In these preliminary results, Papilocare® shows a significant difference in repairing HPV-cervical lesions at 3 months versus control; these findings need to be confirmed upon study completion.
EFFICACY OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL TO CLEAR HPV. INTERIM ANALYSIS RESULTS

J. Cortés 1, A.C. López 2, S. González 3, L. Serrano 3, S. Palacios 4, D. Dexeus 5, C. Centeno 6, Y. Gaslaim 7

1Private Practice (Palma de Mallorca) (Spain), 2Hospital Quironsalud (Málaga) (Spain), 3Centro Médico Gabinete Velázquez (Madrid) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women´s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain), 7Procare Health SL (Barcelona) (Spain)

Background / Objectives

To evaluate the efficacy of Papilocare® -a Coriolus versicolor-based vaginal gel- to clear HPV at 6 months.

Methods

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US or LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment. Preliminary analysis of percentage of patients with HPV clearance at 6 months in both total and high risk genotype virus population are presented. HPV genomic evaluation has been centrally-conducted in IECM laboratory (Lugo, Spain). Papilocare® arms (A+B) were combined for this analysis.

Results

Data about HPV clearance from 26 patients are available. HPV clearance was observed in 56% of patients using Papilocare® (n=16) vs 30% in control group (n=10) (p=0.247; Fisher test).

High risk genotypes viruses were detected in 18 patients. At 6 months, 50% of patients in Papilocare® group (n=12) showed HPV clearance vs 17% (n=6) in control group (p=0.315; Fisher test).

Conclusion

In these preliminary results, Papilocare® shows a positive trend in HPV clearance at 6 months, especially in high risk genotype virus population; these findings need to be confirmed upon study completion.
USE OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL IN PATIENTS WITH PRECANCEROUS HPV LESIONS. INTERIM ANALYSIS RESULTS

J. Cortés 1, A.C. López 2, S. González 3, L. Serrano 3, S. Palacios 4, D. Dexeus 5, C. Centeno 6, J. Combalia 7

1Private Practice (Palma de Mallorca) (Spain), 2Hospital Quironsalud (Málaga) (Spain), 3Centro Médico Gabinete Velázquez (Madrid) (Spain), 4Instituto Palacios de Salud y Medicina de la Mujer (Madrid) (Spain), 5Women’s Health Institute (Barcelona) (Spain), 6Clínica Diatros (Barcelona) (Spain), 7Procare Health SL (Barcelona) (Spain)

Background / Objectives

To evaluate the efficacy of Papilocare®-a Coriolus versicolor-based vaginal gel- to repair cervical mucosa in women with HPV-related cytology alterations and consistent colposcopy image.

Methods

A randomized, open-label, parallel-group, controlled clinical trial. Currently recruiting 96 positive-HPV women age 30 to 65 with pap result of ASC-US, LSIL or AG-US and concordant colposcopy image, randomized into 3 groups: A) Papilocare® 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months; B) Papilocare® 1 cannula/day for 3 months + 1 cannula/alternate days for 3 months; C) no treatment as usual practice. Interim analysis of secondary endpoints - changes in epithelialization of the cervix evaluated by standard colposcopy and in perceived stress evaluated by PSS14 - are presented. Papilocare® arms (A+B) were combined for this evaluation.

Results

Data from 47 patients at 3 months and 29 patients at 6 months are available. 20.7% and 47.5% of patients in Papilocare® group improved the cervix epithelialization at month 3 and 6 respectively vs 22.2% and 16.7 in control group (p=ns). A trend to stress reduction vs basal was observed in the treatment group at month 3 (-0.9 points) and was significant at month 6 (-2.9; p=0.045, Student’s t-test). Patients in control group showed a trend to stress increase at month 3 and 6 (+0.5 and +4.7; p=ns). There were not significant differences between treatment groups.

Conclusion

In these interim analysis results, Papilocare® shows a positive trend in cervix epithelialization and a significant stress reduction; these findings need to be confirmed upon study completion.
EFFECT OF A NON-HORMONAL CORIOLUS VERSICOLOR VAGINAL GEL AMONG POSITIVE-HPV WOMEN WITH NO COLPOSCOPY CERVICAL LESIONS. A PILOT STUDY.

S. González 1, L. Serrano 1, C. Emsellem 2

1Centro Médico Gabinete Velázquez (Madrid) (Spain), 2Procare Health SL (Barcelona) (Spain)

Background / Objectives

To evaluate the effect of a Coriolus versicolor-based vaginal gel (Papilocare®) on cervical epithelialization in positive-HPV women with no colposcopy lesions.

Methods

An exploratory, prospective, observational study. Sexually active positive-HPV women aged > 25y with negative pap and no colposcopy cervical lesions were included during routine clinical visits and treated with Papilocare® once daily for 21 consecutive days. Primary endpoint: change vs baseline in epithelialization degree of the cervix mucosa evaluated by standard colposcopy and rated by investigator from 5 = No ectopy o 1= severe ectopy and bleeding. Secondary endpoints: 1) changes in vaginal signs and symptoms evaluated by likert-type scale from 7= severity to 28= absence, 2) changes in vaginal microbiota evaluated by pyrosequencing and 3) patient satisfaction.

Results

21 patients were included. Papilocare® showed a positive trend to improve the re-epithelialization of the cervix: mean score improved 20% (3.79 vs 4.47 baseline vs final; T test p<0.006). 52.6% of patients improved cervix epithelialization and a score of 5 was observed in 63% of women. A trend to improve symptoms was observed despite of few symptoms at baseline: 71% of patients reached maximum symptoms score at the end of treatment period. Eight patients improved the symptoms score and 3 worsened. A “moderate/complete satisfaction” and some degree of «positive impact on wellness» were reported by 84% and 90% of evaluated patients, respectively. Vaginal microbiota analysis is currently ongoing.

Conclusion

In this pilot study, Papilocare® shows promising benefits in the variables analyzed; these findings need to be confirmed in a larger study.
PAPILLOPLEX™ HR HPV – A NOVEL MULTIPLEX ASSAY FOR DETECTION AND GENOTYPING OF ALL 14 HR HPV TYPES IN A SINGLE CLOSED-TUBE REAL-TIME PCR REACTION

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Background / Objectives

Multiplex Probe Amplification (MPA) is a patented real-time PCR-based technology allowing detection and genotyping of up to 20 different targets in a single closed-tube reaction, thus significantly increases throughput capability. Papilloplex™ HR HPV is a CE IVD marked product for qualitative detection and differentiation of all 14 high-risk HPV types in a single analysis.

In the present study, we carried out a comparative analysis of the performance of Papilloplex™ HR HPV test with other well established assays on clinical samples. Analytical specificity of the assay was also interrogated using the WHO HPV LabNet proficiency panel.

Methods

The Papilloplex™ HR HPV test was applied to 500 disease enriched cervical liquid based cytology samples obtained from the Scottish HPV Archive, Edinburgh, with known concurrent pathology results. Samples were also tested using the Abbott RealTime High Risk HPV assay, the Qiagen digene HC2 HPV DNA Test, the Diamex Optiplex HPV Genotyping kit and Roche Linear Array® HPV Genotyping Test. Concordance between the comparator assays and Papilloplex™ HR HPV was performed using both binomial and McNemar’s test.

Results

The Papilloplex™ HR HPV was able to detect and genotype high risk types 16, 18, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68a and 68b in a single reaction. The limit of detection for the assay was up to 5 genome copy numbers for HPV 16 and 18 in WHO LabNet samples with 100% accuracy for genotyping. No significant difference in the qualitative detection of high risk HPV was observed between the Papilloplex™ HR HPV and the four assays described above. Type-specific concordance was also high.

Conclusion
In conclusion, this study shows that the Papilloplex™ HR HPV is efficient in combined screening and genotyping of HPV DNA. The current commercially available probe-based methods are limited to detection of only one target sequence per fluorescence channel. The MPA technology overcomes this limitation, allowing 14 targets to be detected and quantified in a single closed-tube reaction.

These data indicate that the analytical performance of Papilloplex™ HR HPV is comparable to established HPV assays at the level of generic high risk HPV detection and at the type-specific level. The assay shows potential promise from both disease management and epidemiological perspectives.
P08-02
Development and validation of HPV test intended for use in cervical cancer screening “AmpliSens HPV HCR screen-titr-14-FL”

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Background / Objectives

By now, molecular diagnostics of HPV is not implemented in cervical cancer screening program in Russian Federation. Nonetheless, HPV testing becomes more and more popular among clinicians, gynecologists and oncologists. Thus, need for modern screening test consistent with new scientific data and acceptable under restricted conditions has emerged.

Methods

“AmpliSens HPV HCR screen-titr–14-FL” assay allows quantifying of 14 most oncogenic HPV types with simultaneous typing of 16, 18, and 45 types in one tube. By amplifying two HPV genome region (E1/E6) the assay can distinguish non-integrated and fully integrated forms of 16, 18 and 45 types.

For clinical validation 900 samples were tested in compare to HC2 test (Qiagen). Among them 100 specimens diagnosed HSIL/CC and 800 – NILM/LSIL. Besides them, 6246 samples were tested to establish clinically meaningful cutoff based on HPV viral load. The samples were obtained during screening testing in commercial laboratories and oncological centers of Russian Federation.

Conclusion

Considering samples tested against comparator test there was 97.7% overall agreement, an 96.4% positive agreement, kappa value 0.94 (95% CI 0.92 – 0.96). Negative cytology specimens showed 97.8% overall agreement, 93.3% positive agreement, kappa value 0.93 (95% CI 0.89 – 0.96). For abnormal cytology (only 45 specimens) there was 97.7% overall agreement, 100% positive agreement, kappa value 0.95 (95% CI 0.87 – 1).

Considering clinical samples, specimens with HSIL/CC diagnose have mean viral load 5.9lg (CI 95% 4.8 – 7.0). In 228 NILM/LSIL samples mean viral load was 4.5 lg (CI 95% 2.1-6.9 lg). The cutoff was established by 3.0 lg per 10⁵ epithelial cells with clinical sensitivity 98.6% (CI 95% 88.8 – 98.6) and clinical specificity 87.5% (CI 95% 85.2-89.9).
Mass Spectrometry as a Reliable High Throughput Technology for Routine HPV Diagnostics

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Background / Objectives

About 90% of cervical cancer is caused by the infection with special subtypes of Human Papilloma Virus (HPV).1 High-risk HPV tests were recently approved by the U.S. Food and Drug Administration (FDA) as a primary screening tool for cervical cancer risk in women aged 25-65 years without a simultaneous Pap smear. Molecular test systems are required to detect high- but also low-risk HPV subtypes with high specificity and sensitivity in a time and cost effective manner. MALDI-TOF Mass Spectrometry System (MassARRAY®, Agena Bioscience, Inc.) has the potential to meet these requirements.

Methods

The HPV MassARRAY® panel detects 19 specific oncogenic HPV genotypes in one single multiplex reaction. We analyzed 10 liquid based cytology samples and compared the results with the RT-PCR based COBAS and the hybridization based HPV LCD-array system.

Results

All high risk HPV subtypes detected by the COBAS system or the HPV LCD-array were also identified by MassARRAY®. Whereas the COBAS system detected a maximal number of 3 HPV types (16, 18 and twelve other genotypes without subtyping), the MassARRAY® and the HPV LCD-array could discern further HPV subtypes in several patients.

Conclusion

We conclude that the MassARRAY® HPV assay represents a highly specific, sensitive, reliable and cost-efficient method for the detection of HPV subtypes in liquid samples (and FFPE samples 2) in a high throughput setting.

References

P08-04
COMPARISON OF mRNA AND DNA HPV LEVELS IN HRHPV-POSITIVE PRIMARY SCREENING SAMPLES USING DIGITAL DROPLET PCR

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Background / Objectives

HPV infection is the known cause of cervical cancer and in Sweden, the recommendation for primary HPV testing in cervical cancer screening was initiated in 2016. In the Örebro region, the Aptima (Hologic) HPV assay is used for primary HPV testing, detecting mRNA from 14 hrHPV genotypes, however without distinction between types and also without a human control gene for verification of sample adequacy. The Aptima assay is an in vitro amplification test for qualitative detection of E6/E7 viral messenger RNA (mRNA) but the expression level in a sample might not always correlate with the magnitude of a positive assay signal, especially for samples near the assays detection limit. Using the sensitive digital droplet PCR method we aim to compare mRNA and DNA levels in clinical samples as well as establishing laboratory cut-off levels that can be used as internal controls.

Methods

This ongoing study includes hrHPV positive samples from the primary HPV screening in Örebro, Sweden. Analyzed Aptima sample tubes are collected and 200 µl of residual sample extracted for sample DNA. Genotyping using extracted DNA is performed with Anyplex II HPV28 (Seegene). For HPV16, -18, 33 and 45 positive samples, corresponding liquid based ThinPrep cytology (LBC) vials are retrieved and used for both RNA and DNA extraction. Digital droplet PCR is performed in parallel for both DNA and mRNA amplicons using primer and probesets for E6/E7 of genotypes 16, 18, 33 and 45, also including the human control gene HBB.

Results

Results will be presented at congress.

Conclusion

Results will be presented at congress.
Population-based HPV Testing Performance: Comparison HC2 and Cervista HPV Testing Assays

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Background / Objectives

Human papillomavirus (HPV) and Pap cytology co-testing has been used in our institution for cervical cancer screening and post therapy surveillance. In our patient population, HPV testing performance by Hybrid Capture 2 (HC2, Qiagen, Valencia, CA) or Cervista HPV assay (Hologic Inc., Bedford, MA) is limited.

Methods

We retrospectively searched our institution’s database for women aged 30 years or older who underwent HPV/Pap cytology co-testing in our Cancer Prevention Center (CPC, for general screening population) or Gynecology Clinics (GYN, for cancer surveillance). HC2 HPV assay (2007-2010) or Cervista HPV assays (2011-2016) were used in both clinics. A total of 16,214 women from CPC (mean age, 55 years; 30-91 years) and 10,588 women from GYN clinics (mean age, 51 years; 30-96 years) were included in the study. HC2 and Cervista HPV assays were compared by HPV testing results stratified by Pap test results in both clinics. The differences were analyzed by Fisher’s exact test. A total of 175 follow-up biopsies from women who visited GYN clinic and had HSIL/carcinoma Pap results were reviewed. The sensitivity for predicting high-grade cervical intraepithelial lesion or carcinoma (CIN3+) was calculated for these women with HC2 or Cervista HPV assays.

Results

In the CPC, HPV positive rates were significantly different between Cervista HPV (4.3%) and HC2 (3.4%, P=0.006) in women who had a Pap result of Negative for Intraepithelial Lesion or Malignancy (NILM). HPV positive rates were similar between HC2 (6.1%) and Cervista HPV assay (6.8%) in women with NILM Pap results in the GYN clinic (P=0.21). In the GYN clinics, HPV positive rates were moderately different between Cervista HPV (25.8%) and HC2 (20.7%) in women with Abnormal Squamous Cells of Undetermined Significance (ASC-US) Pap result (P=0.02). In women with High-grade Squamous Intraepithelial Lesion (HSIL) or carcinoma Pap results, HPV positivity was significantly lower by Cervista HPV (78.8%) than HC2 (92.4%) (P=0.008). However, no significant difference of the sensitivity of HPV to predict CIN3+ was observed between Cervista HPV (81.8%) and HC2 (89.2%) (P=0.30) in women with HSIL/carcinoma Pap results.
Conclusion

In a low-risk cervical cancer screening population, increased HPV positivity by Cervista HPV testing in women with a NILM Pap test may result in a more frequent follow up for women with NILM Pap results. The efficacy of the Cervista HPV assay is marginally lower than that of HC2 in women with HSIL/carcinoma in a cancer surveillance population. Further studies are needed to delineate the efficacy of both Cervista and HC2 HPV assays in this population.
SIGNIFICANTLY HIGHER RISK FOR HIGH-GRADE CERVICAL LESIONS IN FOLLOW-UP BIOPSY ASSOCIATED WITH POSITIVE APTIMA HPV TESTS THAN COBAS TESTS

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Background / Objectives

HPV tests and genotyping have been used in clinical risk assessment. The purpose of this study was to analyze the performance of two common HPV testing platforms in risk evaluation for high-grade cervical lesions (HSIL+, including CIN2 and above).

Methods

Between January 1 and December 31, 2016, 4732 Pap tests with biopsy confirmation were analyzed along with HPV tests performed on Cobas (Cobas 4800 system, Roche Molecular Diagnostics, Pleasanton, CA) or Aptima (Hologic/Gen-Probe, San Diego, CA) platforms.

Results

There were 4105 and 627 Pap samples tested on Cobas and Aptima platforms, respectively. Both platforms were highly sensitive for biopsy-confirmed HSIL+ lesions (98% and 96% for Cobas and Aptima, respectively, p=0.51). However, Cobas HPV testing showed significantly higher positive rates for diagnosis of benign (86% vs. 56%) and LSIL (90.5% vs. 66.4%) on biopsy as compared to Aptima. As a result, Aptima HPV testing had a significantly higher specificity for HSIL+ than Cobas (38% vs. 12%, p<0.0001). Overall, performance of Aptima platform was superior to Cobas in detecting biopsy-confirmed HSIL+ due to providing significantly higher positive predictive value (25% vs. 16%, p<0.0001) and overall accuracy (48% vs. 24%, p<0.0001). Aptima hrHPV genotyping also demonstrated a significantly higher specificity for HSIL+ on biopsy than Cobas genotyping measured by either HPV 16/18/or45 (87% vs 73%, p<0.0001) or non-16/18/or45 (51% vs. 39%, p<0.0001) with comparable sensitivity (50% vs. 52%, p=0.68 and 47% vs. 46%, p=0.92, respectively).

Conclusion
Despite Aptima and Cobas platforms offer comparably high sensitive tests for high-grade lesions, Aptima HPV test and genotyping demonstrated significantly higher specificity and positive predictive value than Cobas testing for biopsy-confirmed HSIL+ lesions. The considerable difference may be related to the significant increase in E6/E7 expression following HPV DNA integration in HSIL+ lesions. The significantly higher specificity and overall accuracy of Aptima test and genotyping for HSIL+ lesions may be useful in clinical risk management by identifying high-risk populations.
Background / Objectives

HPV DNA test is performed in a cytology laboratory by trained cytotechnologist as a triage test after borderline cytology or in follow-up after excisional treatment of cervical intraepithelial neoplasia. The aim of the study was to analyze the application of the HPV test in routine cytology laboratory practice in the detection of patients with increased risk of HSIL.

Methods

We retrospectively analyzed the results of 19,459 HPV tests (Hybrid Capture 2, Qiagen, Germany) performed between 2005 and 2015 and compared with cytological diagnosis on conventional Pap smear. We also analyzed data of initial cytology and HPV test result from 1157 patients in a six-year follow up period after HPV test was made. A positive outcome represents a histological diagnosis of HSIL +.

Results

Out of the 19,459 HPV tests, 41.9% were positive, of which 21.5% of negative cytology, 39.5% of ASCUS, 71.6% of ASC-H, 77.3% of LSIL, 86.2% of HSIL, 16.9% of AGC and 75% of AIS. Out of 25 cases of cytological diagnosis of cervical cancer HPV test was negative in two histologically verified cervical cancer and in five cases of endometrial cancer. Of 1157 patients with HPV test made in 2009, 652 (56.4%) had abnormal cytology, and 473 (40.9%) had positive HPV test. The mean age of patients was 37 years (range 16-77 years). In the six-year follow up period histological analysis was performed in 213 patients and verified HSIL + in 173 patients which is 25% of all initial abnormal cytology and 34% positive HPV tests HSIL + was found in 2.5% of patients with initially negative cytology, 8.3% of ASCUS, 10.9% of LSIL, 10% of AGC, 56.5% of ASC-H, 63.5% of HSIL and in 100% of cytological diagnosis of cancer. For all the tested samples, the HPV test showed 94.2% sensitivity, 68.4% specificity, 34% positive predictive value and 98.5% negative predictive value. Reflex HPV testing in the triage of ASCUS showed 86.2% sensitivity, 65.9% specificity, 18.7% positive predictive value and 98.1% negative predictive value. In the six-year follow up with the initial ASCUS cytology in 8.3% cases verified HSIL + lesions, and with additional triage HPV testing this percentage
rises to 18.7%.

**Conclusion**

The percentage of positive HPV test increases with the severity of cytologic diagnosis. The high negative predictive value confirmed the value of the test in the triage of borderline cytology. Knowledge of cytologist and clinician of the positive HPV test may improve the selection of patients with an increased risk of HSIL lesions.
A HIGHLY EFFICIENT ASSAY FOR DETECTION OF HIGH-RISK HPV E7 PROTEINS IN CERVICAL SAMPLES

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Background / Objectives

Persistent infection with high-risk human papillomavirus (hrHPV) types is a prerequisite for development of cervical dysplasia and cancer. During progression, deregulation and overexpression of viral proteins E6 and E7 occur, leading to loss of cell cycle control and neoplastic transformation. Current cervical cancer screening methods rely on cytological analyses compromised by frequent false-negative results and thus low sensitivity. HPV DNA-based tests pick up frequently infections without underlying disease leading to a low specificity. A more effective and reliable screening approach may involve exploitation of the oncoproteins E6 and E7 for specific detection of cervical dysplasia.

Methods

A hrHPV E7 sandwich ELISA – recomWell HPV 16/18/45 - was developed for detection of the hrHPV types 16, 18, and 45. Suitable for measurement of E7 protein are liquid-based cytological samples in PreserveCyte.

Results

Sensitivity (CIN2+/CIN3+/CxCa: 36.1/58.3/85.7%), specificity (>98%), positive predictive value (PPV) (CIN2+/CIN3+/CxCa: 59.5/56.8/16.2%) and negative predictive value (NPV) (>97.5%) were calculated across all studies with 1572 clinical samples.

1473 samples were analyzed for validity of E7-based triage for HPV16/18 positive women. 282 women were positive for hrHPV DNA testing and further subjected to colposcopy. For the detection of CIN2+ for HPV16/18 positive women without further triage, sensitivity and PPV were 100.0% and 11.11%, respectively. No triage of HPV16/18 positive women required 9 colposcopies to diagnose one case of CIN2+. The sensitivity of recomWell HPV16/18/45 was 100.0% (meaning that no CIN2+ case was missed) and PPV was 19.75%. The recomWell HPV16/18/45 identified all 16
CIN2+ cases, requiring 43.75% less colposcopies than no triage of HPV16/18 positive women.

**Conclusion**

Detection of hrHPV E7 by ELISA is a feasible method for diagnosing HPV-induced, high-grade cervical dysplasia. Our results support the detection of HPV E7 oncoprotein as a method of triage to colposcopy for HPV16/18 positive women (instead of no triage) in the framework of a screening program based on primary HPV screening with HPV 16/18 genotyping.
Background / Objectives

The Xpert HPV test (Cepheid) detects HR-HPV 16, 18-45 and 3 groups of other HPV types (P3 : 31-33-35-52-58, P4 : 51-59 and P5 : 39-56-66-68) by PCR, including the detection of a reference gene, confirming an adequate cellularity. To date this technique hasn’t yet been evaluated for cervical smears fixed in BD-SurePath transport medium.

The HC2 (Qiagen) HPV test targets 13 HR-HPV types (no HPV 66 detection).

The aim of the study was to evaluate the performance of the Xpert HPV test on SurePath fixed cervical smears by comparing it to the HC2 HPV test as reference method.

Methods

We tested 110 consecutive SurePath ASCUS smears by HC2 and Xpert HPV using the residual cell pellets after BD-autocyte® PAP cytology.

Samples with discordant results were submitted for detailed genotyping by Inno-LIPA PCR Version Extra II (Fujirebio).

We further evaluated the repeatability (5x) with different technicians on 2 different modules(4 HPV+ and 1 HPV- smears), the stability across time of one positive smear at 1, 7, 14 and 28 days and the reproducibility between the initial vial and the residual cell pellet (35 samples).

Results
Of the 110 HC2 tested ASCUS smears, 57 were HC2 HR-HPV+, 53 were HC2 HR-HPV-. The overall concordance was 89.1%, the negative concordance was 96.2% (two HC2- smears were Xpert HPV+) and the positive concordance was 82.5% (of 57 HC2+ samples, 10 were Xpert HPV-).

Among these 10 HC2+/Xpert HPV- smears genotyping revealed: one HPV 39+, one HPV 52/66/70+, one HPV 33/53+, one HPV 33/35/51/58/53/70/52/44+ and six not targeted HPV types: four HPV53+ samples, two of them coexpressing HPV54 or HPV67, one HPV67+ and one not yet classified HPV type.

Among the HC2-/Xpert HPV+ cases one was HPV 18/66/70/62+ and one HPV 16/51/62+.

The repeatability according to different technicians/modules and across time was 100%. The reproducibility between initial vials and cell pellets was 97%.

When we look at the 10 HC2+/HPV- cases and considering the targeted HPVs, after genotyping only 4 Xpert HPV- cases were true false negatives, all of them with a RLU/cut off ratio < 5 considered at low risk for CIN2+(1). None was false positive.

The other 6 HC2+/Xpert HPV- concerned non targeted HPVs due to cross-reactions of HC2 with low risk and potentially HR-HPVs. Colposcopy was normal for 5 of these 6 patients, 2 of them with a biopsy within normal limits.

Two HC2-/Xpert HPV+ smears were probably related to insufficient sample quantity.

Our global, negative and positive concordances (89.1%, 96.2% and 82.5%) show a performance quite similar to the ones reported for the PreservCyt (Hologic)(2,3).

Conclusion

The SurePath transport medium is suitable for routine HPV testing with the Xpert HPV.

References


HR-HPV TESTING ON FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) SAMPLES: PERFORMANCE EVALUATION OF XPERT® HPV VERSUS PCR INNO-LIPA® EXTRA II GENOTYPING AND P16 IHC ON 28 HEAD AND NECK CARCINOMAS

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Background / Objectives

Today the HPV status of oropharyngeal carcinomas is a prognostic marker impacting treatment.

The Xpert®HPV test (Cepheid) detects HR-HPV 16, 18-45 and 3 groups of other HPV types (P3: 31-33-35-52-58, P4: 51-59 and P5: 39-56-66-68) by PCR including the detection of a reference gene, confirming the presence of an adequate number of cells. To date only one publication used this technique on FFPE tissue sections (1).

The aim of the study was to evaluate the Xpert HPV test on FFPE tumor samples by comparing it to the INNO-LIPA PCR Version Extra II (Fujirebio) genotyping and p16 immunohistochemistry (IHC) as reference methods.

Methods

The Xpert HPV-test on FFPE was validated on six 4 µm FFPE tissue sections of ten cervical biopsies (5 CIN2/3 and 5 within normal limits) and 5 anal biopsies with AIN 2/3 compared to p16 staining.

Tissue sections were deparaffinised, followed by a simple lysis (ATL lysis buffer, Qiagen) with proteinase K for 4 hours, heated for 1 hour at 90°C then diluted in 1 ml ultra-filtered water and processed.

Four of the positive samples were tested at different lysate dilutions: 1/2, 1/4, 1/8, 1/16 and 1/32.

We then performed Xpert HPV tests on 28 FFPE tumor samples of head and neck carcinomas formerly tested by INNO-LIPA PCR (13 HR-HPV positive and 15 negative) and compared them to the IHC expression of p16. Only an intense diffuse staining of > 80% of tumor cells was considered positive, patchy staining was considered negative.
Results

All high grade cervical and anal neoplasias were Xpert HPV and p16 positive.

For the 4 diluted CIN2/3 lysates, HR-HPV was still detected at a dilution of 1/32 and two 4µm sections showed to be sufficient.

Of the 28 head and neck tumor samples, 13 were INNO-LIPA HR-HPV positive, 15 were negative. The overall concordance was 85.7% with a negative concordance of 93.3% and a positive concordance of 76.9%.

One Inno-Lipa negative sample was Xpert HPV as well as p16 positive. Among 13 INNO-LIPA positive samples, 3 were Xpert HPV negative, p16 staining was negative favoring a non viral carcinogenesis. These cases could reflect a possible latent HPV infection in the vicinity of the tumors, detected by the INNO-LIPA PCR.

Conclusion

In all samples tested the overall concordance between Xpert HPV and p16 IHC was 100%.

Xpert HPV testing is feasible without DNA extraction even on very small tissue samples and may be considered as a valuable method for the detection of HR-HPV in FFPE tissues.

References

HPV TESTING FOR CERVICAL CANCER SCREENING: EXPERIENCE IN CENTRO MEDICINA LABORATORIAL GERMANO DE SOUSA/HOSPITAL CUF DESCOBERTAS

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Background / Objectives

Human papillomavirus (HPV) is a well-studied etiologic agent for cervical cancer dysplasia and neoplasia. Worldwide the most common HR-HPV are 16/18, and approximately 70% of cervical cancer are due to these genotypes. The HPV test, as primary method of cervical cancer screening, decreases the incidence of invasive carcinoma in 60-70%, and its performance is superior when compared to cytology, showing a negative predictive value very close to 100% [Consenso SPG, 2014]

HR-HPV screening is highly sensitive, but specificity depends on subsequent evaluation strategies and screening frequencies. Various methods are available for HPV detection and FDA-approved assays are on the market using either signal or target amplification methodologies. The aim of this study was emphasizing the overall performance of the methods used by Centro Medicina Laboratorial Germano de Sousa (CML GS) and correlate the results with cytological examinations in a 5 years sample population from Hospital Cuf Descobertas using different molecular platforms.

Methods

From January-2012 to Decemeber-2016 were analyzed more than 6000 cervical samples by HPV-molecular and conventional-cytology methods. HPV-molecular methods used where: Hybrid-Capture2; Cobas-HPV test; Clart Human papillomavirus 2; PapilloCheck.

The cytological results were registered with SNOMED nomenclature.

Conclusion

This study will contribute for a better understanding of the wide spectrum of HPV infection and provide held information to establish interpretation algorithms in diagnostic management. The results obtained to the incidence and most frequent type of HPV were in agreement with the results particularly described by the Portuguese Society of Human Papillomavirus [Consenso SPG, 2010].

The most frequent HPV HR type in Portuguese population is HPV53, where the malignancy rate is not as high as 16/18, but a shift possibility can occur with
universalization of vaccine. The hc2 and 16/18-Cobas accomplished concordance in false positive rate, detection rate and specificity. However some statistically significant differences were seen, particularly 16/18-Cobas yield lower false negative rate for Abnormal Cytological results, subsequently higher negative predictive value. For those reasons 16/18-Cobas testing should be better for triage. When choosing of any HPV assay for cervical screening, quality control and quality assurance aspects should also be considered, in order to maximize the potential of each method in the diagnostic algorithm.
In-house liquid based medium validation for hrHPV detection with Hybrid Capture 2 (HC2), QIAGEN

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Background / Objectives

In Slovenian cervical cancer screening program ZORA, Qiagen HPV test Hybrid Capture 2 (HC2) is used for a triage of women with low-grade cytology and as test of cure since 2011. For these analyses cervical samples are collected in Standard Transport Medium (STM), Qiagen. The major disadvantages of STM are poor preservation of cell morphology and high cost. In-house liquid based medium (LBM) is already extensively used in routine laboratory practice for immunocytochemical and molecular tests at the Institute of Oncology Ljubljana and in some other Slovenian laboratories. It is cheaper than STM and enables both morphological and molecular analysis. Routine use of in-house LBM in the national cervical cancer screening programme would allow a single sampling procedure for both liquid based cytology and HPV testing. However, the new medium might affect results of hrHPV analysis. The aim of the study was to compare and validate in-house LBM toward the STM for detection of hrHPV with HC2.

Methods

In 183 women (aged from 20 to 64 years, 38.3 on average) referred to colposcopy two cytological cervical samples were taken prior colposcopy by physician at the colposcopy clinic. First cytological sample was taken with endocervical brush and Ayer spatula for PAP smear, after that both devices were stored in in-house LBM; second was taken with Qiagen brush and stored in STM. Cytological samples in different media were analysed on hrHPV by HC2 at cut-off value RLU/CO = 1.0. Results were compared and then validated against the worst histology result from the screening registry within one year since the samples were collected.

Results

HPV-positivity rate was higher in in-house LBM (135/183, 73.8%) than in STM (128/183, 69.9%). Agreement of results was excellent (174/183, 95.1%; Kappa = 0.879 (p < 0.001). Test performance was comparable, however STM had slightly higher sensitivity for CIN2+ (95.5 vs. 96.6%) as well as specificity (46.8 vs. 55.3%), NPV (91.7 vs. 94.5%) and PPV (63.0 vs. 67.2%). Among 9 discordant cases, 1 case was HPV negative in in-house LBM but positive in STM and 8 cases were HPV positive in in-house LBM but negative in STM. 89/183 (48.6%) women had CIN2+.

Conclusion
Comparable HPV-positivity rate, agreement of HPV analyses, sensitivity, specificity, PPV and NPV for CIN2+ between the two media suggest that in-house LBM could be used for hrHPV testing instead of STM to reduce costs and preserve morphology. However, larger prospective study on screening population has to be performed to confirm this assertion.
P08-13
ONCLARITY IN THE DIAGNOSIS OF PATIENTS WITH CERVICAL LESION: COMPARISON WITH HC2 AND LINEAR ARRAY

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Background / Objectives

Many methods are available today for HPV testing: they differ for technology, targets, and information on the genotypes detected. A key issue is represented by the differences in analytical and clinical sensitivity, especially in case of genotyping. Aim of this work was the evaluation of BD Onclarity HPV assay in a group of patients referred to the European Institute of Oncology of Milan for a cervical lesion.

Methods

One hundred sixty-seven women scheduled to be conservatively treated for a CIN2+ lesion were enrolled. For all the patients a cervical sample was taken before treatment, and the results of Qiagen Hybrid Capture 2 and Roche Linear Array HPV Test, cytology and histology were available. BD Onclarity was performed on a leftover aliquot.

Results

Concordance of HC2 and Onclarity was 92% (150/163), with 13 samples giving discordant results (4 hc2 negative and Onclarity positive – 2 CIN3 and 1 Carcinoma histology- 9 hc2 positive and Onclarity negative – 3 CIN1 and 6 CIN3 histology (2 of which were also Linear Array negative)). Looking at genotyping a complete concordance was found in 75.5% (126/167 of the cases), reaching the 86% when adding the samples partially concordant in case of multiple infections.

Conclusion

This study performed in a group of women with a high prevalence of disease showed a good concordance between HC2 and Onclarity in the cervical samples taken before treatment. Regarding genotyping the comparison with Linear Array confirmed a good concordance between the two methods.
Comparison of Seegene Anyplex II HPV 28 detection and Abbott Realtime High Risk HPV test on NOVAprep liquid-based cytology media.

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Background / Objectives

High Risk (HR) HPV DNA testing is a highly sensitive method to screen women at risk of CIN2+ lesions. But many assays and various media for cervical sample collection are available. So, we aimed to compare the Seegene Anyplex™ II HPV 28 Detection and the Abbott RealTime High Risk HPV test on cervical samples collected on NOVAprep® liquid based cytology media (Novacyt).

Methods

Samples were collected on NOVAprep® media from July 2016 to February 2017. Cytology was performed with NOVAprep® liquid-based cytology platform for cervical cancer screening. Samples with ASCUS cytology were routinely tested with the Abbott assay for HPV testing (14 HR HPV detected:16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Among them, 162 samples were randomly selected for testing with the Seegene assay after extraction on Easymag (Biomérieux) following manufacturer’s recommendations. Anyplex™ II HPV28 Detection simultaneously detects 19 HR HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 9 low-risk HPV (6,11, 40, 42, 43, 44, 54, 61, 70), with semi-quantitative analysis (+, ++ or +++). Discordant results were confirmed by testing again both assays at the same time and then will be sequenced by NGS.

Results

All the 162 samples have interpretable results as internal control was valid in both assays. Global HR HPV prevalence was more elevated with Seegene assay (49.38%) than Abbott assay (37.65%). Regarding the 14 HR genotypes detectable by both assays, overall agreement between both assays was very good (93%; kappa 0.86). All discordant results were confirmed in second runs (Seegene and Abbott), showing excellent reproducibility of each assay. Among the 10 Abbott HR HPV negative samples detected as HR HPV by Seegene, analysis of Abbott amplification curves showed that HR HPV (other than HPV16 or HPV18) were detected in 2 samples after 32 Ct (detection threshold determined by Abbott). For both samples, semi-quantification from Seegene assay found a small amount of HR HPV (only one +). Among these discordant results, HPV 68 is the less efficiently detected HPV by Abbott.
assay (6/10). Small amounts of HR HPV (one +) were also found in three of the four other discordant results. Only one HR positive with Abbott assay was not detected by Seegene assay. Complete analysis of discordant results by NGS is ongoing.

Conclusion

NOVAprep® medium previously validated with Hybrid Capture assay has demonstrated again strong performances in terms of detection of HR HPV and stability of the patient samples with Abbott RealTime High Risk HPV test and Seegene Anyplex™ II HPV 28 Detection assays.

References


Background / Objectives

Study of diagnostic significance of methylation status of WIF1 gene and microRNA expression (Mir 92a, 22, 25) in diagnosis of SIL and cervical cancer.

Methods

Clinical (including colposcopy); fluid cytology with agent staining using BD Sureph T.M. method for immunocytochemical (ICC) examination with p16/Ki67 dual labeling; HPV test by means of RT-PCR method, WIF1 gene methylation; microRNA expression (Mir 92a, 22, 25). Final diagnostic verification of SIL and cervical cancer has been carried out on the basis of histologic examination.

101 patients aged from 18 to 49 years have been tested (average age 32.7±0.5 years).

The 1st group – 31 patients with LSIL; the 2nd group – 26 patients with HSIL; the 3rd group – 12 patients with squamous cervical cancer (SCC); the 4th group – 32 healthy patients negative for intraepithelial lesions or malignancy (NILM).

Results

Correlation analysis of morphological examination and cancer markers p16 and Ki67 has shown direct, strong and significant correlation between these two methods (r-Pearson = + 0.7 p = 4.75 × 10-17 for Ki67; r-Pearson = + 0.83; p = 1.8 ×10-29 for p16).

Correlation analysis between the findings of morphological examination and WIF1 gene methylation has established direct, strong, significant bivariate correlation between these two methods (r-Pearson= + 0.8; p = 7.0 ×10-33), besides correlation analysis between microRNA expression has demonstrated direct, significant correlation between MiR92a (r-Pearson = + 0.27; p=0.007).

Conclusion

We have revealed significant correlations between the findings of morphological examination confirming the diagnosis of SIL and squamous cervical cancer; cancer
markers p16 and Ki67; WIF1 gene methylation; and microRNA expression. All above-mentioned methods can be used in complex diagnosis of SIL and cervical cancer.
P08-16
DIAGNOSTIC EXCISION OF CERVIX IN WOMEN WITH PERSISTENT HPV INFECTION WITH NO FORMER EVIDENCE OF CIN IN CYTOLOGY

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Background / Objectives

A persistent infection with human papilloma virus (HPV) is identified as a main risk factor for cervical cancer. Further investigation with cytology and colposcopy has been shown to have lower sensitivity than HPV-testing to diagnose CIN2+. In this study we performed a diagnostic excision of the transformation zone (TZ) by loop electrosurgical excision procedure (LEEP) in women with persistent HPV infection with normal Pap-smear to evaluate the eventual proportion of histologically confirmed CIN2+ in the specimen.

Methods

We prospectively recruited 91 women with persistent HPV-infection without any abnormalities in cytology. In total 40 women attended a gynecological examination including repeated HPV test, Pap smear, endocervical cytology, colposcopy with biopsies and diagnostic LEEP. Biopsies and the LEEP specimen was subjected to histologic analysis. The HPV test was performed using a multiplex real-time PCR assay (hpViR) as earlier described, which detects the following high-risk HPV types 16,18,31,33,35,39,45,51,52,56,58 and 59 (18 and 45 are detected together, and 33,52 and 58 as one group).

Results

In 19/40 women the HPV infection still persisted at the study visit and 32% (6/19) of those women had CIN2+ in histology of the LEEP specimens. All the cytological samples were normal and none of the punch biopsies confirmed CIN2+ in these women. Of the 21/40 women who had cleared their HPV infection at the study visit all but one with CIN 1 had normal histology of the LEEP specimen.

Conclusion

Our results highlight the high risk of undiagnosed CIN2+ in women with persistent HPV infection combined with a normal gynecological examination, Pap smear, endocervical cytology and colposcopy with biopsies. In such cases LEEP must be kept as a diagnostic and treatment option, at least in women without future desire for
pregnancy. Counseling women about the risks and expected effects of the treatment can help them to do an optimal informed choice.
Are non-vaccine replacing vaccine genotypes in young women targeted by vaccination programs? A trend analysis from opportunistic screening in Luxembourg

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Background / Objectives

While widespread human papillomavirus (HPV) vaccination is likely to reduce the prevalence of vaccine types 16 and 18, it remains unknown whether high-risk nonvaccine genotypes will fill this ecological niche. In Luxembourg where the national vaccination program introduced in 2008 achieved a coverage of approximately 60%, the first cohorts of vaccinated girls are now entering opportunistic cervical cancer screening yielding an opportunity to investigate both hypotheses of vaccine type reduction and nonvaccine genotype replacement.

Methods

We extracted HPV test results from a large clinical laboratory in Luxembourg offering HPV genotyping in the context of opportunistic cervical cancer screening. 17901 HPV test results of cervical samples of adult women (mean age 37 y.) during the period January 2010 – June 2017 were assessed in this study. After screening by Hybrid Capture 2 (Qiagen, Germany) assay, positive samples were genotyped using LCD-Array (Chipron, Germany). We compared fractional polynomial prevalence trends of individual 13 high risk HPV (hrHPV) genotypes by logistic regression and the relative contribution of the 13 genotypes over time in young age groups targeted by vaccination (<25 y.) and older untargeted age groups (>=30 y.).

Results

Overall, 3631 samples (20.3%) were positive for hrHPV, including 583 samples (31.8%) in women younger than 25 y. Increasing prevalence over time (p<0.05) were observed for individual genotypes 39, 51, 52 and 59 among women younger than 25 y., but not in women older than 30 y. Among hrHPV positive women younger than 25 y., the relative contribution of vaccine types 16 and 18 dropped from 34% in 2010 to 7% in 2017 (chi2-test, p<0.001). In this age group, the relative contribution of genotypes 39, 51, 52 and 59 increased from 21% in 2010 to 52% in 2017 (chi2-test, p=0.001).

Conclusion

Between 2010 and 2017, a significant change of hrHPV genotype distribution in young women undergoing opportunistic cervical cancer screening occurred in
Luxembourg. Whether these changes represent genotype replacement remains unclear. Studies in other settings are warranted to verify our findings. In any case, the clinical impact for screening of nonvaccine high-risk genotypes deserves further investigation.
NEW SCENARIOS OF HPV SCREENING - GEORGIAN EXPERIENCE

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Background / Objectives

The study aimed to pilot the modern approach to cervical cancer screening program, which means: a) the application of liquid based cytology, and b) human papillomavirus (HPV) genotyping.

Methods

1500 cervical cytology samples and 1800 blood samples have been analyzed in country of Georgia. The cytology samples had been collected and processed by the usage of materials and equipment provided by Hologic. Prepared smears were post-fixed in 96% ethanol and stained accordingly with Papanicolau protocol. The Bethesda 2001 system terminology was employed for reporting and diagnoses of cervical smears. The blood samples have been collected and processed by the usage of reagents provided by Norgen Biotek for the aim to reveal and genotype HPV. The polymerase chain reaction has been performed.

Results

The negative for intraepithelial lesion or malignancy (NILM) category was equal to 1341 cases (89.40%). Other categories in decreasing order were atypical squamous cells of undetermined significance (ASCUS) with 120 cases (8.00%), low grade squamous intraepithelial lesion (L-SIL) with 9 cases (0.60%), high grade squamous intraepithelial lesion (H-SIL) with 2 cases (0.13%), atypical squamous cells, cannot exclude high grade intraepithelial lesion (ASC-H) with 24 case (1.6%) and atypical glandular cells of undetermined significance (AGUS) with 4 case (0.27%). Cellularity was lower in liquid based cytology (LBC) as compared with conventional smears (CS). Also, nuclear overlap was significantly less observed compared to CS. The smear background was notably cleaner and cell morphology was better evaluated in LBC. In terms of Trichomonas and Candida detection, LBC was superior compared to CS. Doderlein lactobacilli were seen in significantly lesser amounts and were mainly situated in close vicinity to the squamous epithelial cells. Due to lack of pretreatment, the degree of inflammation was better assessed in CS.

The prevalence of HPV DNA has been observed in 586 cases (32.56%). In 320 cases (54.61%) have been determined oncogenic (16/18/31/33/53) HPV.

Conclusion

Our experience shows that LBC is superior to CS in the evaluation of cell morphology and detection of certain microorganisms such as Trichomonas and Candida. The
degree of inflammation is better assessed with CS. CISH is effective and easy for implementation method for HPV genotyping on cervical smears. There has been revealed that HPV genotyping is the effective and accurate screening method.

References


AGE DISTRIBUTION AND RISK PROGRESSION OF HSIL AND HIGHER LESIONS IN THE PUERTO RICAN POPULATION IN 2015

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Background / Objectives

Screening patterns are changing constantly and most of them do not take into consideration younger patients. Most of the High-grade Squamous Intraepithelial Lesions (HSIL) carries a higher risk of progression to cervical cancer given that 40% to 50% of HSIL progress. The aim of this study was to demonstrate the importance of giving relevance to these patients and that age is also a factor for the risk of disease progression, since LSIL patients are not aggressively treated in these younger patients.

Methods

A total 1,072 samples with HSIL or higher lesions were obtained from a pool of 32,620 samples with a dysplasia diagnosis out of a total of 227,946 patients from OB/GYN clinics around Puerto Rico in 2015. The samples were rescreened and classified following TBS criteria for HSIL or higher and then divided by age. Out of the 1,072 samples, 186 of them were tested to determine the risk of progression for overexpression of HPV E6/E7 mRNA, using the OncoTect assay test (IncellDxTM), and was classified as positive in cases where >2% of cells showed overexpression of E6/E7 mRNA.

Results

From these samples, 17.3% of the cases were positive using the OncoTect assay test (IncellDxTM). From 1,072 HSIL or higher cytology samples, the age distribution were: ≤25 (16%), 26-35 (34%), 36-45 (22%), 46-55 (12%), and ≥56 (17%), were 50% of the cases fell under 35 years of age, while the progression risk was: ≤25 (17%), 26-35 (38%), 36-45 (23%), 46-55 (8%), and ≥56 (15%).

Conclusion

This study demonstrated that a relevant percentage (50%) of the patients with HSIL or higher lesions was in the range of 35 years or less, showing that this group had the same percentage of progression to that of older patients. Knowledge that a HSIL or higher lesions prevalence in younger women is by no means negligible, transient or productive HPV infection in this group age is prevalent. Although cytology interpretation has its limitations, it is always best to add more knowledge towards qualifying reports. It is necessary to take early care of these younger patients with HSIL or higher lesions and positive for overexpression of HPV E6/E7 mRNA in the
long run, so as to reduce the risk of progression of disease in the Puerto Rican population.
ACCURATE DETECTION OF HUMAN PAPILLOMAVIRUSES BY PNA MEDIATED REAL TIME PCR USING MELTING CURVE ANALYSIS

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Background / Objectives

Cervical cancer, which is caused by infection with oncogenic human papillomavirus (HPV) is the fourth most common cancer among women in worldwide. In particular, viruses 16 and 18 are known to account for about 70% of the causes of cervical cancer. Although the Pap smear is used as a primary method for cervical cancer screening, it is not possible to predict the potential risk of cancer due to the virus because it tests for cell deformation. Therefore, the need for HPV DNA testing for the early diagnosis of cervical cancer is increasing.

Methods

A new peptide nucleic acid (PNA) - assisted melting curve analysis technique is developed. Each genotype-specific PNA probe, which is conjugated with a fluorescent dye and a quencher, is used as a reporter in a real-time PCR reaction. A PNA probe can design relatively shorter binding sequence than DNA probe, so PNA probe can avoid sequence variation position on a gene. Furthermore, PNA probe showed bigger melting temperature difference than DNA probe when reporter probe bound to a single mismatch target. So, sequence variants on a target gene are easily distinguishable using melting curve analysis. Therefore, PNA-based reporter probe is very useful for multiplex detection in real-time PCR platform to identify a target gene with many sequence variants.

Results

We have developed accurate and simple method to detect of HPV types within 3 hrs in one-tube. PNA probe-based fluorescence melting curve analysis technology in a real-time PCR system [PANA RealTyper™ HPV Screening Kit] is possible to detect 16 types of HPV [Genotyping types: 6, 11, 16, 18(Type associated with vaccine prescription) and Screening type: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68(HPV high risk)]. The PNA probes were designed to detect all variant genes for each HPV type. Using standard materials, each type of HPV identified the sensitivity of detection was $5 \times 10^1$ ~ $5 \times 10^3$ copies. And there was no cross reaction with other HPV types.

Conclusion

PANA RealTyper™ HPV Screening kit is easily and rapidly can be detected HPV types, and it will be a useful and efficient method for detection and discrimination of HPV types in clinical diagnosis.
P09-04
COMPARISON OF P16/Ki67 DUAL IMMUNOCYTOCHEMICAL STAINING, HPV TESTING AND CYTOLOGY RESULTS OBTAINED IN THREE CYTOPATHOLOGY LABORATORIES PARTICIPATING IN SLOVENIAN CERVICAL CANCER SCREENING PROGRAM ZORA


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Background / Objectives

Slovenian organized, population based cervical cancer screening program ZORA is cytology based. Women with low grade (LG) cytology are triaged with HPV testing. After the implementation of the program in the year 2003 the incidence of cervical cancer decreased for almost 50%, from 20.7 to 11.4 per 100,000 women (crude incidence rate). Despite the good results the gradual introduction of HPV primary screening is currently discussed in Slovenia and therefore the pilot study was conducted to compare the results of PAP test, p16/Ki67 dual immunocytochemical staining (ICS) and HPV testing within the Slovenian cervical cancer screening program.

Methods

In 129 women referred to colposcopy, cervical smears were obtained for cytological examination, p16/Ki67 dual ICS (CINtec PLUS test, Roche) and HPV testing (Hybrid Capture 2, Qiagen). Each PAP smear and ICS slide was evaluated blindly in 3 laboratories participating in Slovenian cervical cancer screening program by a screener and a cytopathologist in the same way as it is current practice in the program. Cytology was reported according to Bethesda classification and p16/Ki67 according manufacturer recommendations. Sensitivity and specificity for CIN2+ were calculated for LG cytology (ASCUS+), high grade (HG) cytology (HSIL+), p16/Ki67 dual ICS and HPV testing. For cytology and p16/Ki67 dual ICS summary estimates of sensitivity and specificity were calculated for the three laboratories participated in the study.

Results
The sensitivity for LG cytology, HG cytology, p16/Ki67 dual ICS and HPV testing were 69.2% (95% CI 54.1%–81.1%), 44.4% (95% CI 33.6%–55.8%), 88.2% (95% CI 82.7%–92.1%) and 96.8% (95% CI 89.0%–99.6%) respectively. The highest sensitivity was obtained for HPV testing, however the sensitivity for p16/Ki67 dual ICS staining was much better than for cytology. The specificity for LG cytology, HG cytology, p16/Ki67 dual ICS and HPV testing were 67.2% (95% CI 56.4%–76.5%), 93.0% (95% CI 87.6%–96.1%), 73.1% (95% CI 66.5%–78.8%) and 59.1% (95% CI 46.3%–71.0%) respectively. The highest specificity was obtained for HG cytology and p16/Ki67 dual ICS.

Conclusion

Our results were similar to the results of other studies and support the idea that the introduction of HPV primary screening with p16/Ki67 dual ICS or cytology triage could give better results than cytology primary screening with HPV triage. However, additional larger prospective study on the screening population must be carried out before the policy of cervical cancer screening program in Slovenia would be changed.
Background / Objectives

As jurisdictions prepare for HPV-based cervical cancer screening, programs cannot ignore operational concerns, such as compliance with colposcopy referral for timely disease detection. Colposcopy programs traditionally prioritize women with high-grade cytological abnormalities. With HPV-based screening and cytology triage, traditional patterns for colposcopy prioritization may need to be re-assessed. We present colposcopy compliance and procedure wait times by referral result from HPV FOCAL, a large primary HPV testing RCT.

Methods

HPV FOCAL compared primary HPV testing with liquid based cytology (LBC) triage (for HPV positives) every 4 years to LBC screening every 2 years. Women 25-65yrs (n=18,948) were randomized to the control (CTRL) and intervention (IA) arms. IA: baseline HPVpos received reflex LBC and were referred to colposcopy if >ASCUS; if baseline HPVneg or <CIN2, exit trial at 48mos. CTRL: baseline ASCUS receive reflex HPV and referred to colposcopy if HPVpos. Baseline >LSIL were directly referred to colposcopy; those baseline LBCneg or <CIN2 were rescreened with LBC at 24mos; if LBCneg or <CIN2, exit trial at 48mos. Both arms co-tested with HPV/LBC at 48mos and referred to colposcopy if positive on either test. To enhance colposcopy compliance and standardization, colposcopy procedures occurred primarily at two high volume clinics.

Results

Overall trial colposcopy compliance was 96% within 12 months of referral compared to the provincial program rate of 86%. At 48mos, where both arms received HPV/LBC co-testing, the shortest median wait times from referral to procedure were in those HPVneg/>HSIL (3.6mos) and HPVpos/>HSIL (3.7mos). Time to colposcopy for >HSIL patients, irrespective of HPV outcome was significantly shorter than other referrals; median wait time, >HSIL: 3.7mos, other: 4.7mos (p<0.0001). At 48mos, the largest number of CIN2+ (42%) was detected in those HPVpos/NILM. The trend for prioritization by cytology regardless of HPV positivity was also observed in Round 1 of the trial. Trial colposcopy clinics reported confusion regarding how to prioritize HPV positive results accompanied by low grade or normal cytology.
Conclusion

Trial colposcopy compliance for HPV FOCAL was high (96%) compared to program rates (86%), and median wait times for any referral result were less than 6mos. However, longer wait times were observed in those HPVpos/NILM, where the highest burden of CIN2+ was detected at 48 months. As programs plan for HPV-based screening with cytology triage, protocols for prioritizing colposcopy procedures may need to be re-evaluated based on the combination of both HPV and cytology results.
POSITIVE PREDICTIVE VALUE OF HPV SCREEN TESTS AND HPV 16/18 GENOTYPING AT BASELINE AND 48 MONTHS IN THE HPV FOCAL TRIAL

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Background / Objectives

Evidence suggests that positive screening test performance declines upon subsequent screening in women with a history of negative HPV and Pap co-tests1. We examined the positive predictive value (PPV) of the hybrid capture 2 (HC2) and cobas HPV screen tests, and cobas genotyping at the baseline and 48 mo. exit screens in the HPV FOCAL Trial.

Methods

HPV FOCAL is a randomized trial comparing liquid-based cytology (LBC) to high-risk (hr) HPV for cervical cancer screening. Of 9,552 women randomized to the Intervention arm, 9,514 had valid baseline HC2 and cobas results (cobas was blinded at baseline). Round 1 colposcopy referral was based on baseline HC2 positive together with LBC ≥ASCUS or 12 mo. persistent HC2 positivity. At 48 mo. exit, 8,330 women had valid HC2, cobas and LBC results (no blinding at exit), of whom 7,664 were baseline HC2 and cobas negative. Colposcopy referral at 48 mo. was based on HC2 positivity, LBC ≥ASCUS or cobas HPV 16/18 positivity. We calculated PPV for each screen test and for cobas genotyping at both screening rounds.

Results

PPVs for CIN2+ and CIN3+ at Round 1 and 48 mo. exit are shown in the table. At Round 1, cobas HPV 16/18 positive women had significantly higher CIN2+ and CIN3+ PPVs vs. other HPV test results; PPVs for HC2, cobas and cobas non-16/18 hrHPV were similar. At 48 mo. exit, all CIN2+ and CIN3+ PPVs were lower than at Round 1. For cobas HPV 16/18 positive women, CIN2+ and CIN3+ PPVs were higher, but no longer significantly, than other HPV test results.

<p>| CIN2+ and CIN3+ PPV (95% confidence interval) at Round 1 and 48 Mo. Exit Screens |
|---------------------------------|-------------|-------------|-------------|-------------|</p>
<table>
<thead>
<tr>
<th>Test result</th>
<th>Round 1</th>
<th>Round 1</th>
<th>48 Mo. Exit</th>
<th>48 Mo. Exit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN2+ (n=149)</td>
<td>0.19 (0.17-0.23)</td>
<td>0.10 (0.07-0.14)</td>
<td>0.03 (0.01-0.06)</td>
<td></td>
</tr>
<tr>
<td>CIN3+ (n=68)</td>
<td>0.09 (0.07-0.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC2+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN2+ (n=30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN3+ (n=9)</td>
<td></td>
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</tr>
</tbody>
</table>
Conclusion

At the baseline screen, cobas HPV 16/18 positive women had significantly higher PPVs for CIN2+ and CIN3+ than other HPV test results, but at 48 mo. exit the PPVs for cobas HPV 16/18 positives were no longer significantly higher. As expected, due to lower CIN2+ and CIN3+ prevalence at 48 mo., all PPVs were lower than at baseline. Further research will be required to assess the ongoing utility of screening and triage approaches at subsequent screening rounds following HPV implementation.

References

HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS WITH NEGATIVE HPV TESTING

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Background / Objectives

It is demonstrated that all cervicovaginal squamous cell carcinomas are associated with HPV infection. Based on this evidence, the new international guidelines recommend HPV-DNA testing as the main primary screening method to filter those patients who may present pre-malignant lesions and need to go through further explorations. However, a subset of high-grade squamous intraepithelial lesions is found in women with negative HPV testing.

Our objective with this study is to report the patients in our base data with HPV-DNA negative test and high-grade squamous intraepithelial lesions (HSIL). HPV subtype and histology of the biopsy specimens are reported and taken into account for the final results.

Methods

A retrospective review of the cases identified as citology-positive and HPV-negative testing over a 36 month period at a tertiary care gynecologic center. Two types of testing were compared, enzyme-linked immunosorbent assay (ELISA) detection versus polymerase chain reaction (PCR) of the virus.

Results

A total of 1043 cases (740 patients) meeting the study criteria were selected. 10 patients with high-grade lesions and negative ELISA detection were identified (representing 1.35%), of whom 8 had high risk HPV detected with PCR study (80% of the selected cases).

Of the ten selected patients one of them presented a biopsy positive for vaginal intraepithelial neoplasia (VaIN). The PCR test presented a coinfection with HPV 42 subtype (low risk) and 73 subtype (high risk).

Considering the other nine cervical displasias, three of them presented a biopsy positive for squamous cell carcinoma, with PCR testing positive for HPV subtypes 52, 31, and 73 (high risk subtypes).

Conclusion

As we know, co-testing with the combination of Pap cytology and HPV DNA testing (HPV 16/18) is the preferred cervical cancer screening method for women between
30-65 years old. This combined method has a 5.5% false negative rate in most of the studies.

If we compare the most important international studies with our data we present a lower rate of false negative results using an ELISA testing combined with Pap cytology, and we can overcome this low rate if we complement the testing with a PCR study in the cases of discrepancy between both testings.

We consider that these cases are the ones that could have a greater benefit with the co-testing screening versus an HPV testing alone, as we would be able to compare the citology results with the DNA testing so as to avoid the loss of control and study of those patients with high grade lesions and negative HPV testing.
P09-08
RANDOMIZED HEALTH CARE POLICY EVALUATION OF ORGANISED PRIMARY HPV SCREENING OF WOMEN AGED 56-60


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Background / Objectives

To implement and reliably evaluate primary HPV screening in an established and routinely running organized, large-scale population-based screening program.

Methods

Participants: Resident women in the Stockholm/Gotland region of Sweden, aged 56-60 years were randomized to either i) screening with cervical cytology, with HPV test in triage of low-grade cytological abnormalities (old policy) or ii) screening with HPV testing, with cytology in triage of HPV positives (new policy).

Outcome: The primary evaluation was the detection rate of cervical intraepithelial neoplasia grade II or worse (CIN2+).

Results

During January 2012 - May 2014, the organized screening program sent 42752 blinded invitations with a pre-booked appointment time to the women in the target age group. 7325 women attended in the HPV policy arm and 7438 women attended in the cytology arm. In the new policy, the population HPV prevalence was 5.5%, using an accredited HPV test (Cobas 4800). HPV16 prevalence was 1.0% (73/7325) and HPV18 prevalence was 0.3% (22/7325). In the HPV-policy arm, 78/405 (19%) HPV-positive women were also cytology positive. There were 19 cases of CIN2+ in histopathology, all among women who were both HPV-positive and cytology-positive. The PPV for CIN2+ in this group was 33.3% (19/57). In the cytology policy, 153 women were cytology positive and there were 18 cases of CIN2+ in histopathology. Both the total number of cervical biopsies and the number of cervical biopsies with
benign histopathology was much lower in the HPV policy (49 benign, 87 total versus 105 benign, 132 total).

**Conclusion**

Primary HPV screening had a similar detection rate for CIN2+ as cytology-based screening, already before follow-up of HPV-positive, cytology-negative women with new HPV test and referral of women with persistence.
EVALUATION OF THE IMPACT OF THE hr-HPV BASED CERVICAL CANCER SCREENING: RESULTS OF A FOUR-YEARS EXPERIENCE IN A SINGLE SCREENING CENTER OF ITALY.

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Background / Objectives

The knowledge on the etiological role of high-grade Human papillomavirus (hr-HPV) have caused a radical change in the cervical cancer prevention program by introducing hr-HPV test instead of PapTest as a primary test in the screening program. The aim of this study was to evaluate the impact of the hr-HPV test in the cervical cancer screening in the Latina district (Italy) over a four-year period.

Methods

The population was divided in two groups: the hr-HPV as primary test was performed only on women aged 35-64, followed by a PapTest as triage, while women aged 25-34 were invited to perform only a PapTest.

Results

5.6% of women presented with a positive hr-HPV test and 4.1% presented with a positive PapTest. In the group of women aged 25-34, the hr-HPV test was used as triage for women with ASCUS and 69.9% presented with a positive test. The PPV for high cervical intraepithelial lesions (CIN2+) was higher in women aged 35-64 (9.9% vs 6.9%), while the DR for CIN2+ lesions was higher in young women (2.4 ‰ vs 1.2 ‰). Moreover, we found that 52.5% of women hr-HPV+/PapTest- resulted hr-HPV+ at 1-year recall and the DR for CIN2+ lesions of this population was very low (0.27‰).

Conclusion

Our data confirms that the application of Italian guidelines showed high level of performance; moreover, our data confirms that the application of hr-HPV test in the management of ASCUS leads to a decreased of inappropriate colposcopy due to transitory infection in young women. Finally, because of the small number of CIN2+ lesions, it may be useful to extend the period of follow-up for women hr-HPV+/PapTest- to reduce the number of unnecessary colposcopies due to transitory infections.
ORGANIZED CERVICAL CANCER-SCREENING PROGRAM IN BRAZIL: BARRETOS EXPERIENCE IN 18 MUNICIPALITY OF SÃO PAULO STATE


Background / Objectives

Background: Cervical cancer remains as an important problem for public health authorities in developing countries due to the high rates of incidence and mortality. This malignancy is the second most common cancer among women worldwide, accounting for more than 520,000 new cases and the deaths of approximately 270,000 women, annually. Estimates by the Brazilian National Cancer Institute (INCA) indicate an incidence of 16,340 new cases of cervical cancer for 2016 in Brazil. Objectives: To demonstrate the implementation of an organized cervical screening program in low-resource settings supported by Barretos Cancer Hospital (BCH) initiative to implement.

Methods

Methods: We developed an organizational, laboratorial and human resources training necessary to administrate the program. A computational program to report all epidemiological, clinical and laboratorial findings, and also to trace all necessary informations to periodically recruit the women for regular screening was developed by the BCH. Women from rural and remote areas were screened in mobile units.

Results

Results: More than 160,000 Pap tests were analyzed and 2,900 colposcopy examinations were performed in one single year. Importantly, from 2011 to 2015, 89.4% of all carcinomas were detected at clinical stage in situ carcinoma and I, and only 5% at stages III and IV. In 2014, e.g., 1,130 patients were referred for colposcopy: 98% of the patients from Barretos region attended the call; 97.1% of the patients from other regions attended in Public Health Ambulatories, and 74% attended in Mobile Units from other Brazilian States.

Conclusion

Conclusions: Since the organized system was implemented, 98% of women attended the recall for colposcopy. However, the main restriction of our program for prevention cervical cancer is still the lack of ideal coverage for this agenda. We did not reach yet the 70% of the women target for this proposal as recommended by the international standards.

References

CERVICAL SCREENING AND RISK ASSESSMENT USING MULTIPLEXED PROTEIN ASSAY

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Background / Objectives

Cervical cancer, the second most common cancer occurring in women worldwide, is the result of infection by sexually transmitted, high risk strains of the human papilloma virus (HPV)[1]. The persistence of infection and subsequent integration of HPV into the human genome results in a number of molecular and cellular changes, which override normal growth control leading to sustained cell cycle progression and subsequent transformation to cervical cancer. Continued discovery of the molecular mechanisms engaged in malignant transformation of the cervical mucosa has lead to the emergence of several candidate cervical cancer biomarkers that could be used to identify HPV-positive patients at the greatest risk for developing cervical cancer and could one day provide the opportunity to identify those patients who are most likely to benefit from therapeutic intervention.

Methods

We present a description and quantitative means for detection and prognostic risk assessment of cervical disease, CIN II or above using multiplexed proprietary biomarkers. Histological and immunological evidence and modeling for several protein markers against disease and normal patient samples is discussed and proposed as a basis for a multiplex quantitative laboratory system and eventual point-of-care (POC) approach to extend cervical screening to underdeveloped regions.

Conclusion

Multiplexing of protein-based biomarkers for cervical disease can improve sensitivity and specificity of cervical screening, providing opportunities for identifying patients at high risk, while expanding the reach of cervical care to areas of greatest need.

References
CERVICAL CANCER AND PRECANCEROUS LESIONS SCREENING IN RURAL AREA’S WOMEN BY HPV DETECTION USING SELF-SAMPLED TESTS


Background / Objectives

Cervical cancer is considered an important public health problem especially in developing countries. In Brazil, some populations, for instance the women who live in rural areas, are most vulnerable to the absence of screening organized programs that can provide an extensive coverage and follow up of suspect cases. The possibility of less invasive tests based on self-sample and performed in a domestic environment can increase acceptance rates on screening tests in comparison to the conventional Pap smear. The aim of this study was to evaluate the feasibility of a cervical cancer screening strategy in women who lived/worked in rural areas by using self-sampled tests to detect HPV offered by the Military Police Team, which is responsible to take care of these regions.

Methods

We performed a cross sectional study with prospective collection data from February 2015 to June 2016. The study was developed by Prevention Department of Barretos Cancer Hospital, Barretos, Brazil in partnership and financed by AMIGOH (Einstein’s friends of Oncology and Hematology) which is one arm of the Albert Einstein Benefit Society. We enrolled 386 woman who were employed or lived in rural areas of Barretos, between the ages of 25 and 78 years old (average=41,3). It was offered to the participants the possibility of self-sampled tests to detect high risk HPV which were after processed using Cobas 4800® System (Roche Diagnostics, Laval, Quebec, Canada). This system can provide the detection of 14 high-risk HPV types in a single analysis. The test was offered to each participant by one member of the Military Police (female soldier). All woman with a positive result (HPV positive) were invited to a colposcopy evaluation.

Results

95,6% of woman interviewed accepted the study and performed the self-sample. The main reasons to refuse the study were: “being afraid of getting hurt” and “not consider herself able to perform the test”. In a total of 340 woman with a valid test, 45 (13,2%)
had a positive result for HPV infection of at least one subtype. The combination of the results (colposcopy evaluation and the pathologic results for HPV positive participants) detected 46.3% benign findings, 41.5% CIN1, 7.3% CIN2/3 and 4.9% with invasive squamous cell carcinoma.

**Conclusion**

The high acceptance of self-sampled test, even when not offered by health professionals, showed the potential of this strategy as a complementary instrument on cervical cancer screening, mainly to populational groups non adherent to the conventional strategies.

**References**

HPV DNA SELF-SAMPLING OFFERS A VALID TOOL FOR CERVICAL CANCER SCREENING IN KINSHASA, THE DEMOCRATIC REPUBLIC OF THE CONGO

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Background / Objectives

OBJECTIVES. To offer women in Kinshasa an alternative tool for cervical cancer screening by self-sampling with detection of high-risk (hr) HPV DNA.

Methods

A total of 190 women living in the municipality of Bandalungwa, aged 29-73, participated in the test. Women were individually informed at home by health care workers about the purpose of the test, the procedure, the follow-up, and eventual therapy. All interventions were free of charge. Samples were collected using EvalynR Brush (Rovers Medical Devices), and were analyzed by the Abbott RealTime High Risk HPV assay in the Laboratory of Molecular Microbiology in Ghent, Belgium. In total 187 samples were analyzed.

Results

Application of the Abbott assay resulted in 170 valid hrHPV determinations. hrHPV could be detected in 19% of these samples. HPV16 and HPV18 were present in 16% an 6% of the hrHPV-positive samples, respectively. The prevalence of hrHPV in this test was about five times higher than the prevalence of (pre)cancerous lesions (Bethesda grade LSIL and higher) found with cytology in comparable groups of women in Kinshasa (1,2). The finding of the rather low percentages of HPV16 and HPV18 is in agreement with earlier findings published for women with HIV-negative or unknown HIV serology (1,2).

Conclusion

Self-sampling is a valuable tool that facilitates access to cervical cancer screening in Kinshasa. Further research is needed to resolve the origin of differences between cytology and HPV DNA results.

References

FOR HIGH-RISK HPV TESTING THE SENSITIVITY OF A URINE SAMPLE EQUALS THAT OF A SELF-COLLECTED VAGINAL SAMPLE

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Background / Objectives

Increasing focus has been added toward self-collected samples as a means to increase the participation in the screening programs for cervical cancer. Urine samples have also been tested for this purpose, but the knowledge on performance is still quite sparse.

In this study, the high-risk HPV status of a self-collected (SC) vaginal sample and a urine sample is being compared to a concomitantly physician-taken liquid-based cytology (LBC) sample. The results of the three HPV tests are being compared to the histological diagnosis of biopsies taken in parallel.

Methods

Women referred to colposcopy at the gynecological departments at Lillebaelt Hospital and Odense University Hospital is being in-rolled in the study. A total of 300 women will be included.

A urine sample and a SC vaginal sample using the Evalyn Brush are performed by the women after a short instruction and just before the medical examination. At the colposcopy an LBC sample and biopsies are taken by the gynecologist. The urine, SC vaginal sample and LBC sample are analyzed for the presence of high-risk HPV using the Cobas HPV test, Roche. The biopsies is diagnosed by a pathologist and used as the gold standard.

At this point 70 women have been included in the study.

Results

Urine and a SC vaginal sample are available from all women, while data from LBC and biopsy samples are currently available from 60 and 58 women, respectively. The results of the HPV test are distributed as follows:

For urine and the SC vaginal samples, 46 were identified as positive in both sample types and 19 as negative. The concordance was 93 % (65/70). For SC vaginal
samples and LBC samples the agreement was 87% (52/60), while for urine and LBC a concordance of 80% (48/60) was found.

The sensitivity of the three sample types was the same (94% and 100% for CIN2+ and CIN3+, respectively). For the specificity differences was observed. At CIN2+ the specificity was 38%, 43% and 57% for SC vaginal samples, urine and LBC, respectively. For CIN3+ the specificity was 33%, 38% and 53% for SC vaginal samples, urine and LBC, respectively. The differences are not statistically significant at this point.

Among the women one adenocarcinoma was identified and for this patient all three sample types were HPV positive.

**Conclusion**

These initial data indicate that the sensitivity of a urine sample is equally good as a SC vaginal sample and comparably to the physician-taken LBC samples. Further data are needed in order to determine whether the specificity of a urine sample and vaginal samples is significantly lower than a LBC sample taken by the physician. The updated data will be presented.
A PILOT STUDY OF COMMUNITY BASED SELF SAMPLING FOR HIGH RISK HUMAN PAPILLOMAVIRUS TEST IN CHINESE POPULATION

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Background / Objectives

Cervical cancer ranks as the fourth most common cancer worldwide in women, and it is the eighth most frequent cancer in Chinese population. High risk (HR) human papillomavirus (HPV) is one of the major causes of cervical carcinoma. More than 99% of cervical cancer cases are related to HPV genital infection, which 69.1% of invasive cervical cancer in Chinese population are attributed to HPV HR-subtype 16 or 18. Cervical cancer screening coverage in female population of aged 30-59 years is only 20.9%. This low coverage attributes to the risk of cervical cancer development upon persistent HPV infections. In this study, we therefore investigate the reliability of vaginal specimen collection by self-sampling device (Qvintip, Aprovix) in Chinese population.

Methods

Women aged 30±7.3 years (mean±SD, n=281) attending local health clinic between March 2017 and June 2017 were enrolled in this study. Vaginal specimens were obtained by self-sampling device Qvintip with instructions provided. The specimens were subjected to DNA extraction followed by HR HPV DNA screening using commercial Real Time qPCR test kit.

Results

Among the 281 self-sampling devices collected, observable vaginal fluid was observed in 99.3% of the Qvintips (279/281). β-globin and another housekeeping gene were used as endogenous controls for the presence of DNA. The positive rate for internal control was 98.6% (277/281). The overall infection rate of HR-HPV was 10.8%.

The self sampling device had good acceptability with easy process of specimen collection. The drying and short-term storage (2 weeks) of vaginal fluid on Qvintips did not hinder the reconstitution of specimens for analyses. This provides the possibility of self-sample taking at home which could be suitable for remote area population and enable clinical screenings in laboratories later on. Hence, the value of self-sampling at home should be further investigated.

Conclusion
Community based self sampling was a reliable way for vaginal fluid collection for HPV DNA screening. Implementations of HPV self sampling using Qvintip for the responder population as a primary screening tool is highly achievable.
THE COST-EFFECTIVENESS OF HPV SELF-SAMPLING FOR NON-ATTENDERS IN A DANISH CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

Cervical cancer screening programs are responsible for declining rates of cervical cancer mortality around the globe. However, incidence rate of cervical cancer remains high in un-screened women not attending screening. In the Capital Region of Denmark (RegionH), 24% of women remain unscreened, accounting for 50% of cancers in the region. To address this challenge, the Copenhagen Self-Sampling Initiative (CSi) pilot program was initiated to test the effectiveness of HPV self-sampling as a strategy to reach unscreened women. The pilot successfully demonstrated that 30% of unscreened women could be brought into the screening program2.

Based on the results of the CSi pilot, the RegionH is now implementing self-sampling as a general offer to screening non-attenders over a period of 3 years. A health economic model was developed to predict the cost-effectiveness of this initiative.

Methods

An Excel-based budget impact model was constructed and calibrated with data from the published literature for Denmark’s population, HPV prevalence, and outcomes. Costs were calculated based on direct data from the CSi pilot2,3. For ages 30–59 years, the model compared two identical cytology primary screening algorithms, of which one offered self-sampling.

Results

At full implementation, over 1 screening cycle, the RegionH could expect to bring an additional 32,050 unscreened women into the screening program utilizing self-sampling. Through this additional coverage, 8% more ≥CIN2 and 32% more cancers would be detected. Based on previous analysis in Denmark, it’s expected that 16% of these CIN2 and CIN3 cases, if left untreated, would progress to cancer1. The total costs of the self-sampling program would be an incremental €21,861. This translates to €14 per woman brought into the regular screening program, €1,728 per ≥CIN2 detected, €21,861 per cancer detected, and €11,733 per cancer avoided.

Conclusion
As self-sampling is currently being rolled out in the RegionH, it’s important to understand the cost-effectiveness of this strategy. This analysis demonstrates that self-sampling is a cost-effective strategy to increase coverage of cervical cancer screening programs, improving outcomes for patients.

References


EVALUATION OF THE ROCHE COBAS® 6800 HPV ASSAY WITH COLLI-PEE® COLLECTED, UCM PRESERVED URINE.

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Background / Objectives

HPV testing in urine has been proposed for monitoring impact of vaccination, follow-up of treatment and/or reaching women not participating in a cervical cancer screening programme. The use of Colli-Pee® (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine. The aim of this study was to check compatibility with Colli-Pee® collected, UCM preserved urine and compare performance of the Roche Cobas® 4800 and 6800 HPV assays. In addition, a pilot on impact of different preservatives on HPV detection onto the cobas® 6800 System was conducted.

Methods

Forty-four Colli-Pee® collected, UCM preserved urine samples were analysed. Thirty-two of these samples originated from a cohort of women participating in a therapeutic HPV vaccination trial. These samples were collected by the participants at home and were send uncooled by postal mail to the University of Antwerp. All samples were characterised by an in-house HPV type specific (TS) qPCR method (UAntwerp, Belgium) and or by the Optiplex HPV genotyping kit (Diamex, Heidelberg, Germany). We further tested 15 urine samples of which 10 were previously positive for HPV 16 and/or 18. These samples were stored for 3 days at RT without preservative, with UCM or with Roche preservative.

Results

This pilot study demonstrates that the Roche cobas® HPV 6800 assay performs well with the Colli-Pee® collected, UCM preserved urine samples. All 44 samples were positive for the Roche beta-globin internal control. Comparing CT values of the cobas® 4800 HPV assay with the cobas® 6800 HPV assay showed that lower CT values were observed for the IC control as well as for the HPV 16, HPV 18 and other HR HPV types in the cobas® 6800 System. Compared to cobas®4800 HPV assay an additional 7 samples were positive for HPV 16.

A correlation between the Ct (cycle threshold) values obtained with Cobas® 6800 HPV and the in-house TS qPCR is observed for human DNA and HPV DNA. This further confirms the compatibility of the Roche assay and the Colli-Pee® collected, UCM preserved urine.
The impact of preservative was most noted on the Internal Control, 5 of the 15 samples without preservative were reported invalid.

**Conclusion**

We confirm that the cobas®HPV 6800 assay is compatible with Colli-Pee® collected, UCM preserved urine. The analytical sensitivity seems to be increased compared to the cobas® 4800 system. The importance of a preservative is reconfirmed.
P11-02
HPV GENOTYPING IN ASC-US CITOLOGY AT RIO DE JANEIRO, BRAZIL

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Background / Objectives

Background: The ASC-US cytological result accounts for more than half of the abnormal cytology results. The detection and typing for HPV DNA is effective and beneficial in the management of ASC-US citology results, because the HPV type have the relationship with the severity of the squamous intraepithelial lesions. Objective: The aim of the study was to analyze the effectiveness of the HPV-DNA tests in the initial approach of women with ASC-US cytology.

Methods

Methods: A cross-sectional cohort study was conducted with 100 women from the city of Rio de Janeiro with ASC-US cytology using liquid-based cytology, a colposcopy procedure and HPV DNA testing.

Results

Results: The median age was 43 years. In 50% of the women were HPV positive. HPV genotyping test results showed that HPV types 51 and 16 were most frequent. 74% (37/50) of the women were infected with only one type, and 26% (13/50) were infected by two or more types. Most (60,9%) of the women with normal cytology were HPV negative, while all the women with severe cytology (LSIL, ASC-H, HSIL) were HPV positive. Most (64,1%) of the women with a normal colposcopy were HPV negative, while all of the women with an abnormal colposcopy (LSIL e HSIL) were HPV positive. There was cytoloscopy concordance among 85% (59/69) of the women both tests normals. Only 30% (6/20) of the women with ASC-US cytology had an abnormal colposcopy. However, it was noted that cytoloscopy concordance was obtained in 54,5% (6/11) of the women with severe cytology (ASC-H, LSIL, HSIL). Four women underwent biopsies with histological findings of NIC2 (2 cases), NIC3 (1 case) and microinvasive carcinoma (1 case).

Conclusion
Conclusion: Based on these data, it can be concluded that HPV DNA testing in the initial screening of women with ASC-US cytology may be an effective strategy.

References

A comparison study of the INNO-LiPA and the Linear Array HPV genotyping tests

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Background / Objectives

In a routine setting we analyzed DNA from 100 samples of various tissues with the automated procedure using the INNO-LiPA HPV Genotyping Extra II kit (Fujirebio, Malvern, PA, US) and compared the results with the performance of the manual, highly validated HPV genotyping protocol for Linear Array (Roche Diagnostics, Pleasanton, California, US).

Methods

Both methods are line probe assays based on PCR amplification of part of the L1 region of the human papillomavirus (HPV) genome. INNO-LiPA HPV Genotyping Extra II employs SPF10 primers and is designed for the identification of 28 different genotypes where the hybridization steps are performed on the Fujirebio automated platform. In contrast, the Linear Array targets 37 genotypes using PGMY09/11 primers and the hybridization steps are highly manual.

Results

Based on our study, the two methods are highly comparable with 80% of the cases exhibiting complete agreement. This agreement was higher for single infections than for multiple infections. Ninety two percent of all samples were at least in partial agreement (concordant or compatible), however this number was higher for cervical (97%), than non-cervical tissues (80%). This discrepancy is best explained by the increased number of failed assays and/or lower sensitivity of the Linear Array compared to the INNO-LiPA method when FFPE samples are genotyped. Better performance of INNO-LiPA on often degraded FFPE samples is a logical outcome of its primer design, since Linear Array requires nearly 7 times longer DNA fragments for potential identification of HPV genotypes, than INNO-LiPA does. Moreover, Linear array was inferior in detecting HPV 52, probably due to its shared probe design.

Conclusion

Overall, the two methods are highly comparable regarding performance of HPV genotyping and although INNO-LiPA kit is more expensive, it requires substantial less hands-on work in addition to being superior in genotyping of low quality DNA often characteristic of FFPE specimens.
EVALUATION OF THE PERSISTENCE OF HPV GENOTYPES IN WOMEN TREATED FOR CIN2+ LESIONS

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Background / Objectives

The follow-up of women treated for CIN lesions includes HPV testing and cytology, with different schedules depending on different guidelines. Today it is not yet clear if looking at the persistence of the genotype identified at baseline (before any treatment) would be of help in selecting those women who may present a relapse, while reassuring those cured by the surgical conservative treatment. Aim of this study was the evaluation of the utility of using a test giving an extended genotyping in the follow-up of patients treated for CIN2+ lesion.

Methods

One hundred and sixty-seven women scheduled to be conservatively treated for a CIN2+ lesion were enrolled. For all the patients a cervical sample was taken before treatment and at first follow-up visit. In all the patients the results of histology performed at baseline and at relapse (when occurred) was available. The presence of HPV DNA was evaluated with the BD Onclarity HPV assay, which allows an extended genotyping, detecting HPV16, 18, 31, 45, 51 and 52 in single, and HPV 33/58, 35/39/68 and 56/59/66 in pool. In all the patients results of cytology, hc2 and Linear Array were also available.

Results

Of the 167 patients, 161 had the Onclarity performed also at the first follow-up visit. A negative HPV test was found in 120 women, while 41 tested positive (25.5%): 14 cases presented different genotypes from baseline, while 27 of 41 (65.8%) showed fully or partially persistence. Nine patients (5.4%) relapsed and Onclarity was performed at baseline and follow up in 7 of them: 6 had persistence of the same genotypes, while 1 patient tested negative not only with Onclarity but also with hc2. In this patient cytology was HSIL, and Linear Array HPV test revealed the presence of HPV18 and 73 at baseline, with the persistence of HPV73 at relapse.

Conclusion
This study showed that the inclusion of the evaluation of the HPV genotype specific persistence may represent a valid option to follow patients treated for CIN2+ lesions. In our study we found that relapses were detected only in patients with persistence of the same genotype detected at baseline.
HPV type specific distribution in women attending routine cervical screening in rural Malawi

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Background / Objectives

Population specific HPV prevalence studies are useful for planning vaccine and screening strategies. Our objective was to assess the prevalence of high-risk (HR) and low-risk (LR) HPV in women attending routine cervical screening in rural Malawi using a new, analytically sensitive genotyping assay.

Methods

Provider-taken and self-taken specimens were obtained between January 2016 and March 2017 from women attending routine VIA (Visual Inspection with Acetic acid) clinics in Nkhoma Hospital and associated Health Centres. All samples had previously been tested using the Xpert® HPV assay. Samples were classified based on VIA and Xpert results into four categories: VIA+/Xpert+; VIA+/Xpert-; VIA-/Xpert+; VIA-/Xpert-.

A multiplex real-time PCR based assay (Papilloplex Any HPV; GeneFirst, UK) was performed which provides individual genotyping in two tubes (14 HR-HPV:16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 56, 66, 68(a&b) and 16 LR-HPV: 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 67, 69, 70, 72, 81, 82). HPV type specific prevalence of HPV in all four categories and concordance with Xpert were assessed.

Results

High concordance was seen in HR-HPV positivity between Xpert and Papilloplex HPV in provider-taken {proportional agreement=0.98 (95% CI- 0.79-0.90), k=0.68 (95% CI- 0.58-0.79)} and in self-taken samples {proportional agreement=0.97 (95% CI- 0.77-0.92), kappa=0.72 (95% CI- 0.57-0.80)}.

HR- and LR-HPV prevalence is summarised in Table 1. HPV 16 was most common in VIA+ women (N=156) followed by 52, 18, 51, 35, 31/33/45. HPV 6/44 then 67/72 were the most frequent LR-HPV. HPV 16 was also most common in VIA- women (N=139) followed by 52, 51, 35, 58 and 31/33 with the most frequent LR-HPV being 6 then 44/67/72/54/61. In women with a VIA assessment of ‘suspicious/frank cancer’ (N=42), HPV 16 was most frequent followed by 18, 45, 52, 31 and 51.
### Sample sets (VIA and Xpert HPV results)

<table>
<thead>
<tr>
<th>Provider-taken</th>
<th>Self-taken</th>
<th>Provider-taken</th>
<th>Self-taken</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIA+/Xpert+</strong></td>
<td>85.18%</td>
<td>82.61%</td>
<td>20.97%</td>
</tr>
<tr>
<td><strong>VIA+/Xpert-</strong></td>
<td>13.79%</td>
<td>4.17%</td>
<td>15.69%</td>
</tr>
<tr>
<td><strong>VIA-/Xpert+</strong></td>
<td>87.10%</td>
<td>80%</td>
<td>25.0%</td>
</tr>
<tr>
<td><strong>VIA-/Xpert-</strong></td>
<td>19.04%</td>
<td>15.38%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

### Conclusion

In both VIA+ and VIA- groups, HPV 16 is the most common HPV type in women attending screening clinics in rural Malawi followed by types 52, 51, 35, 18 and 31. However, HPV 16, 18, 45 are the three most common types found in visually assessed suspicious cancers. High concordance was seen between the two tests but analytically sensitive Papilloplex assay detects higher prevalence of HR HPV prevalence. Further studies on type specific prevalence using analytically sensitive assays are warranted in clinical settings in low income countries.
Background / Objectives

Cervical cancer prevention through primary vaccination is a promising approach especially for countries with high cervical cancer prevalence. Geographical differences of HPV genotype distribution are known, however little data are available for Ethiopia, the second most populated country in Africa.

Methods

Women were recruited as part of a visual inspection with acetic acid (VIA)-based cervical cancer screening program in 2 health centers in the Gondar region of Ethiopia. All consenting women underwent VIA preceded by collection of a cervical specimen for HPV testing using the Hybrid Capture 2 (HC2) assay (Qiagen™). HPV-DNA-positive samples were genotyped applying a bead-based hybridization assay using Luminex technology. All women with abnormal findings were referred to gynecologist for further management.

Results

700 women aged 18-64 years (median 35, IQR 27.40) were enrolled in the study of which 73 (10.4%) were HPV positive.

The most common high risk HPV genotypes were HPV 16 (55.6%), HPV 53* (22.2%), HPV 56* (13.3%), HPV 52 (11.1%), HPV 31 (8.9%), HPV 39* (6.7%), HPV 58 (6.7%), HPV 18 (4.4%), HPV 35* (4.4%), HPV 70* (4.4%) [*not included innonavalentHPV vaccine against types 6, 11, 16, 18, 31, 33, 45, 52, and 58].

Conclusion

These data inform on the discussion regarding use of second versus first generation HPV vaccine for the country. More data are needed regarding HPV genotype distribution and correlation to disease status in Ethiopia.
P11-07
Genotyping of human papillomavirus in triaging of low-grade cervical cytology

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Background / Objectives

Atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intra-epithelial lesions (LSIL) are minor lesions of the cervical epithelium, detectable by cytological examination of cells collected from the surface of the cervix of a woman. Usually, women with ASCUS and LSIL do not have cervical (pre-) cancer, however a substantial proportion of them do have underlying high-grade cervical intra-epithelial neoplasia (CIN, grade 2 or 3) and so are at increased risk for developing cervical cancer. Therefore, accurate triage of women with ASCUS or LSIL is required to identify those who need further management.

The objective of the study was to evaluate whether typing of human papillomavirus among women with low-grade cervical cytology can improve the ability to identify women with cancer or cervical intrepithelial grade II+ (CIN II or worse)

Methods

This is prospective observational study carried out in Germans Trias i Pujol Hospital in Barcelona. A total of 266 women with low-grade cervical cytology participating in the study.

We used residual liquid-based cytology samples for HPV genotyping. Extracted DNA was subjected to parallel polymerase chain reactions using three primer sets for HPV DNA amplification. HPV+ samples were genotyped by DNA sequencing.

During 24 months, we study persistence and evolution of LSIL and ASCUS by citology and colposcopy each 6 months and HPV genotyping each year.

We study the individual and combined risk of progression depending on each HPV

Results

The adjusted prevalence of cervical intraepithelial neoplasia grade 2 or greater in our study was 23.5%.

The odds of persistence and progression were higher in women infected with HPV 16, 18 and 31.
HPV 16 was detected in 40% of cases with CIN II or worse but only among 24% of all tested women. HPV 31 was detected in 20% of cases with CIN II or worse but only 11% among all tested women. Testing the three HPV types with higher risk (HPV16/18/31) detected 71% of CIN II or worse, with 36.9% testing positive. Positivity of other high risks HPV types had decreased risk of CIN III.

Conclusion

HPV genotyping may aid in prognosis of LSIL course. We should include HPV 31 en triaging LSIL, as is the second most frequent HPV type involved in progression.

Different high-risk HPV types confer different risks for the presence of CIN2 or worse, implying that genotyping could be a useful optimization of triaging strategies.

References


HPV L1 GENETIC DIVERSITY VARIANTS IN STRAINS FROM NORTHEASTERN MEXICAN PATIENTS AND THE DISCREPANCY RESULTS OBTAINED BY REAL TIME PCR.

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Background / Objectives

Cervical cancer is the second leading type of neoplasia in Mexican women and high risk HPV has been associated with its development[1, 2]. Variations in HPV genomes have been associated with the severity of cervical lesions [3, 4]. We determined genetic variations founded in L1 region from different HPV types isolated from northeastern Mexican patients and compared them with the results obtained with real time PCR genotyping kit.

Methods

We collected 255 cervical samples from patients who attended colposcopy consultation at the Hospital Universitario “Dr. Jose Eleuterio Gonzalez” of the Universidad Autónoma de Nuevo León in Monterrey, Nuevo Leon, Mexico. One hundred forty-one samples were HPV positive, and genotyped using the E. coli Amplisens HPV HRC Genotype titre FRT kit using the AB7500 Fast Real Time PCR. We detected 43 HPV monoinfected samples and amplified the L1 region using PGMY 09/11 primers and sequenced the PCR product. The obtained sequences were assembled, and posteriorly analyzed with MEGA7. We built a phylogenetic tree with the maximum likelihood method using the GTR +G model [4, 5].

Results

The most frequent HPV was HPV 52. Seventy percent of our samples were infected with more than one HPV type. We identified 43 HPV strains from Mexican patients, were the 14% of those patients had a persistent HPV infection. Most of the patients (41%) presented a clinically valuable viral load. These strains had different genetic variations in the L1 region, and most of them were synonymous. There was no significant association of the detected HPV type with the viral load. Twenty HPV sequences differed from the HPV detected by real-time PCR.
Conclusion

The discrepancy between the HPV type detected by real-time PCR and Sanger sequencing was high [6]. This could be due to coinfections with several HPV types, with higher viral loads. We speculate that the included HPV probes cross-react with other HPVs not included in the kit [7]. Due to the samples background and the high frequency of the HPV coinfections in our population, there is a need to isolate HPV strains to evaluate their carcinogenic potential and the genetic evolution of these viruses circulating in the northeastern region. Even if their viral load was not associated with the HPV type, the genetic variations found could explain the carcinogenic potential these strains might have.

References


P12-01
DIAGNOSTIC VALUE OF HPV16 AND HPV18 VIRAL LOAD AND INTEGRATION STATUS AMONG AFRICAN WOMEN INFECTED WITH HIV

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Background / Objectives

To evaluate the performance of E6 HPV16/18 Viral load, (E6-E2) HPV16/18 Viral load (viral load "integrated") and E2/E6 ratio (integration coefficient to detect cervical high-grade lesions (CIN2 +)) in a cohort of African women infected with HIV enrolled in the HARP (HPV Research Partnership in Africa) project. A total of 1238 women were enrolled in the HARP study. At baseline and at 18 months of follow-up (endline) the following screening tests were performed: HPV test, Cytology, VIA / VILI, colposcopy and biopsy if one test positive. Histology was systematically confirmed by the End Point Committee. In addition, HPV detection and genotyping was performed using the INNO-LiPA HPV genotyping Extra assay. 245 women co-infected with HPV16 and/or HPV18 were included in the present study. Their median (range) age was 35 (25-49) years, CD4 + cell count was 417 (7-830) cells/µL, and 158 (66.4%) women were on ART; 122 positive for HPV 16 and 78 for HPV18 at inclusion; 103 positive for HPV 16 and 66 for HPV18 at 18 months. 25 co-infected at baseline and 11 at endline.

Methods

Quantitative real-time PCR targeting the E6 and E2 genes were performed using serial dilutions of HPV16 and HPV18 plasmids as standard curves. Total cellular DNA was measured by real-time PCR of the GAPDH gene, and the results were expressed as the number of copies of E6 and E2 per 1,000 cells. SiHa, Caski and Hela cell lines were used as controls.

Results

It was observed a very significant (p<0.0001) increase in HPV16 E6 viral load, and HPV16 (E6-E2) viral load and a decrease in E2/E6 ratio as the grade of cervical lesions increased. It was also observed an increase (p=0.029) in viral loads with the lesion grade. There was a strong correlation between viral loads E6 and E2-E6 HPV16 and HPV18 (Rs = 0.899 and 0.962 respectively). E6-E2 and E2/E6 are not independent of E6. E6 viral loads of 4 log copies/1,000 cells for HPV16 and of 2 log copies/1,000 cells were associated with high grade lesions. Sensitivity and specificity
of these viral load levels were 83% and 75% for HPV16, and 50% and 70% for HPV18, respectively.

Conclusion

A high HPV16 viral load (> 4 log E6 DNA copies / 1000 cells) or, to a lesser extent, for HPV18 (> 2 log E6 DNA copies / 1000 cells) is associated with cervical high-grade lesions. Among women without high-grade lesion at baseline, a high HPV16 viral load is associated with progression to high-grade lesion at 18 months, this was not observed for HPV18. E6 Viral load and E2-E2 ratio are not independent markers of viral load E6. HPV16 viral load might constitute a triage test for HPV16-positive women. HPV18 viral load is of more limited interest given the low sensitivity / specificity ratio.
THE USE OF P16/KI-67 DUAL STAINING TECHNOLOGY ON CERVICAL CYTOLOGY OF PATIENTS UNDERGOING A LLETZ PROCEDURE

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Background / Objectives

The main objective of this prospective observational study was to investigate the diagnostic performance of the p16/Ki-67 DST for detecting CIN 2+ in comparison with HR-HPV testing and Pap cytology in a LLETZ referral population. Secondary study objectives were investigation of the diagnostic performance of the DST for triage of patients with persistent low-grade CIN (ASCUS or LSIL cytology results during > 24 months) or an inconclusive colposcopy examination.

Methods

A total of 110 patients referred for a LLETZ were enrolled between October 2016 and mid-March 2017. From each participant, a cervical cytology sample was obtained before the onset of the LLETZ procedure. On each sample, we conducted the DST (Roche CINtec Plus Test ®), Pap cytology and an HPV DNA assay (identifying 17 different HPV types, including the 13 “high-risk” genotypes). Test results were correlated with the cone biopsy result to guarantee excellent disease ascertainment.

Results

The overall disease prevalence of CIN 2+ was 56%. The mean age was 41 years, with 38% of patients being younger than 35 years. P16/Ki-67 positivity increased with histological severity. Positivity was 35% in CIN 0, 46.6% in CIN 1 and 80% in CIN 2 patients. Positivity increased to 95.9% and 100% in cases of a histological diagnosis of CIN 3 or invasive carcinoma. The overall sensitivity and specificity of the DST for detecting CIN 2+ was 94% and 58% respectively with a PPV of 74% and a NPV of 88%. HR-HPV testing results in a similar sensitivity of 92% but considerable lower specificity of 21% compared to the DST. When ASCUS or worse is considered a positive test result, Pap cytology still has the lowest sensitivity of 89% compared to dual staining and HR-HPV testing. In cases of persistent low-grade CIN (n=19), the DST had a non-inferior sensitivity of 100% and superior specificity of 67% for detecting CIN 2+ compared to HR-HPV testing. In cases of an inconclusive colposcopy examination, the DST provides a sensitivity of 95% and negative predictive value of 94% for detecting or excluding relevant disease.

Conclusion
The p16/Ki-67 DST provides high sensitivity and improved specificity compared to HR-HPV testing and Pap cytology for predicting CIN 2+, making it an interesting tool for identifying relevant disease in patients referred for a LLETZ. Test performances were even better in patients referred with persistent low-grade CIN, but conclusions should be drawn with care because of the low number of patients in this subgroup (n=19). In cases of an inconclusive colposcopy examination, the DST seems to provide an excellent negative predictive value for excluding almost all relevant disease.
P12-03
ANALYSIS OF THE INFLUENCE OF P16 IN THE INTER AND INTRA OBSERVER CONCORDANCE IN THE DIAGNOSIS OF INTRAEPITHELIAL NEOPLASIA OF CERVIX GRADE 2 (CIN2)

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Background / Objectives

The histological classification of the pre-neoplastic lesions of the cervix contemplates three categories, cervical intraepithelial neoplasia (CIN) grades 1, 2 and 3. This classification is based on a subjective assessment of the thickness of the affected epithelium: CIN1 is considered the affectation of a third or less, while the thickness of the affected epithelium in CIN3 affects more than two-thirds of its thickness. It is established that the really pre-cancerous lesion is the CIN3. But between CIN1 and CIN3 we have the CIN2, an equivocal diagnosis, given the established very low inter-observer agreement to establish its diagnosis, about 30% according to data available in the literature.

We have today a biomarker, the p16, a marker of viral integration, which can objectify the diagnosis of CIN2 and, consequently, improve its concordance and diagnostic safety.

To assess inter and intra-observer agreement in the diagnosis of CIN 1 - 2 and 3, and to study how the use of the p16 modifies these values.

Methods

We collected 100 biopsies of cervix diagnosed with CIN2 from our records between 1997 and 2007. We performed p16 in all of them.

In a first phase, three expert pathologists evaluated 297 cervix biopsies, including cases of CIN2 randomly inserted along with cases of CIN 1, CIN 3 and invasive carcinoma of the cervix. They have subsequently analyzed p16 separately. In a third phase, the CIN2 biopsies have been passed along with their corresponding p16 for assessment and diagnosis. To conclude, 150 of the 297 biopsies were randomly selected to evaluate inter-observer agreement.

Results

The final results of the work will be presented at the Congress.

Conclusion
The final results of the work will be presented at the Congress.

References


PROTEOMIC COMPOSITION OF CERVICOVAGINAL FLUID IN HPV-ASSOCIATED CERVICAL LESIONS

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Background / Objectives

To determine changes of the cervicovaginal fluid (CVF) proteomic composition for assessment of the severity of HPV-associated cervical lesions among reproductive age women.

Methods

The study involved 30 women with various forms of HPV-associated cervical lesions (ASCUS, LSIL and HSIL). All samples of cervicovaginal fluid were prepared for further proteomic analysis by tandem mass spectrometry (HPLC-MS/MS). Semi-quantitative data analysis including identification and annotation of proteins was carried out using the software package MaxQuant and Perseus.
Results

The protein panels specific to the various forms of HPV-associated cervical lesions (ASCUS, LSIL and HSIL) were identified. The first group of proteins (P4HB, HSPA8, C4BPA and others) characterized the early changes associated with HPV infection and cervical epithelium lesion, including penetration of viruses into the cell and its transcription, impaired function of the complement system. The second group of proteins (PRDX5, YWHAE, LRG1 and others) were directly involved in the development and progression of cervical neoplasia and characterized late changes, in particular, reduced apoptosis, impaired differentiation and maturation of the epithelium, and the transformation of atypical cells.

Conclusion

The protein composition of the CVF was studied to assess the severity of HPV-associated cervical epithelial lesions in reproductive age patients by tandem chromato-mass spectrometry (HPLC-MS / MS). The protein panels, specific for various forms of HPV-associated cervical epithelial lesions are determined (the first group - P4HB, HSPA8, C4BPA, the second group - PRDX5, YWHAE, LRG1).
DETECTION OF CERVICAL (PRE)CANCER ON THE BASIS OF CERVICOVAGINAL FLUID: POSSIBILITIES FOR DEVELOPMENT OF A SELFTEST

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Background / Objectives

Despite tremendous efforts over the last decades, current screening methods for cervical cancer still have limitations in sensitivity and/or specificity. Moreover, vaccines are not effective against all HPV types and efficiency is uncertain in case of previous infection. In the search for more specific and sensitive biomarkers, and additional challenge represents the application of these biomarkers in low- and middle income countries where the incidence of cervical cancer is highest. The Cervico Vaginal Fluid (CVF) is composed of secretions originating from organs that are part of the female genital tract, including vagina, cervix, endometrium and ovaries; hence the proteome of this fluid contains a wealth of information concerning the physiological status of all of these organs. Since many studies have proven self-sampling as a good and acceptable sample collection method for subsequent DNA genotyping, cytology or immunohistochemistry, CVF may very well be suited for the development of a selftest for triage of suspected cases or screening in low- and middle income countries.

Methods

A differential proteomics study on CVF was performed using six CVF samples from healthy and six samples from precancerous women. Extracted proteins were run over a 2D-LC-MS/MS platform and quantified by spectral counting. Lists of identified CVF proteins were analyzed by Ingenuity pathway Analysis (IPA) to find out whether cervix cancer pathways were reflected in the CVF. A series of candidate biomarker proteins was further validated by ELISA or mass spectrometry (MRM).

Results

We identified alpha-actinin-4 (ACTN4) as a protein biomarker that could discriminate between the healthy and (pre)cancerous states with a sensitivity and specificity of resp. 84 and 86%. Based on the list of proteins that were differentially abundant in both types of CVF, a set of cervical cancer protein biomarkers interconnected within several cancer-related pathways was identified by Ingenuity Pathway Analysis (IPA). We quantified these biomarkers by ELISA or mass spectrometry (MRM) in CVF samples from healthy or precancerous woman in order to further increase the discriminative power in combination with ACTN4.
Conclusion

The cervical vaginal fluid may contain several biomarkers which, when used in an appropriate combination, could be used for development of an accurate cervical cancer screening test. Since collection of CVF is non-invasive, these biomarkers allow for the development of a self-diagnosis test to be used for screening, prediction or follow-up of cervix cancer.

References

CLINICAL UTILITY OF p16INK4a AS A DIAGNOSTIC ADJUNCT FOR UNDERLYING CIN2+ CERVICAL LESIONS

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Background / Objectives

In Honduras, premalignant and malignant lesions of the cervix continues to represent a major burden on the health care system mainly due to decreased specificity of screening tests as well as significant interobserver variability in the diagnosis of these lesions. Since p16INK4a is a surrogate marker of HPV E7-mediated pRb catabolism, it has been successfully deployed for the classification of HPV-related disease. This study aimed to assess the clinical significance of overexpression of p16INK4a in cervical lesions.

Methods

To help delineate the utility of p16INK4a, colposcopy-directed biopsy samples drawn from a larger study (n = 20: negative, 9; CIN I, 3; CIN II, 8) were analyzed by immunohistochemistry for expression of p16INK4a. Testing for high-risk human papillomavirus types by Hybrid Capture2 and genotyping by L1 HPV region PCR (GP5/6+) followed by reverse hybridization (LiPA) was performed on concurrent cervical scrape specimens.

Results

None of the negative and CIN I cases (n=12) expressed the p16INK4a protein. On the other hand, all CIN II specimens (n=8) were positively associated with p16INK4a expression and high-risk HPV presence (P < .001), showing a sensitivity and specificity of 100% (95% CI: 75.7-100.0). The HPV prevalence in the negative and CIN I cases was 50% as opposed to 100% of CIN II cases. The viral types identified in the CIN II cases were 16, 18, 35, 58, 51 and 66, being HPV16 the most common.

Conclusion

Although a small sample size, our findings show a possible utility for adjunct p16INK4a in addition to HR- HPV testing to distinguish between negative/low-grade (CIN 1) and high-grade squamous intraepithelial lesions (CIN II+) to avoid overtreatment of false-positive cases and under treatment of false-negative cases. It is suggested to test a larger sample size to increase the statistical significance of the study.
MODERN MULTIDISCIPLINARY MONITORING OF CERVICAL CANCER RISK

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Background / Objectives

Currently, Romania is ranked first in Europe in terms of cervical cancer mortality. In this context, the solution which has been developed in recent years is the secondary use of molecular markers more specific for cervical precancer, combining their high sensitivity with high specificity. Among these methods, p16 / Ki67 dual immunocytochemistry is the most studied.

Methods

One hundred and eighty-three patients who performed the Papanicolaou test, the HPV-DNA test and the immunocytochemistry test (CINtec PLUS) from June 2014 to June 2017 were examined. Patients with the positive CINtec PLUS test were recommended for a colposcopy examination and subsequent biopsy.

Results

The sensitivity and the negative predictive value of CINtec PLUS was 100%, the specificity 75.2% and the positive predictive value 60.2%. Performing a double staining test in patients with ASCUS type cervical cytology changes in the study group has been shown to be very effective in identifying precancerous cervical lesions.

Conclusion

Thus, by using the p16 / Ki-67 immunocytochemical staining, the medical attitude in screening or monitoring young patients with HPV infection, high-risk strain and ASC-US, the results being optimized. Unnecessary colposcopy examinations are avoided (indications of colposcopy are restricted to CINtec PLUS Positive and invasive gestures on nulliparous women are limited. CINtec PLUS negative results are monitored by repeating cytology testing and HPV DNA testing at 12 months.
METHYLATION OF INHIBITORS WNT SIGNALLING PATHWAY AND HPV TYPES IN CERVICAL CANCER


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Background / Objectives

Most cervical cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV). Genetic and epigenetic changes as the silencing of inhibitors WNT signaling pathway can affect the outcome of HR-HPV infection. Considering that the methylation of DNA is important for the carcinogenic process, the aim of this study was to analyze status of methylation of DKK3, SOX17 and SFRP2 genes regarding HR-HPV types 16/18/45, staging, degree of differentiation and origin of cervical cancer.

Methods

A total of 169 paraffin-embedded tissue blocks from biopsies performed in cervical cancer patients were selected. HPV detection and genotyping were performed using the INNO-LiPA HPV Genotyping assay. After treatment with sodium bisulfite, the samples were submitted to MS-PCR

Results

The age of the patients at the time of diagnosis of cervical cancer ranged from 26 to 91 years, with an average of 52.3 years (95% CI = 49.3-54.7). The mean age of the patients who were diagnosed with adenocarcinoma was 46 years (95% CI = 40.7-51.3). Squamous cell carcinoma on average affected 53.9 year old women (95% CI = 49.7-56.2). A total prevalence of HPV was 94.3%. All cases as diagnosed squamous cell carcinoma were positive for HPV among cases of adenocarcinomas, 86.4% were positive for HPV. The methylation of genes was a prevalent event in cervical cancer ranging from 65.5% to 90.0%. The prevalence of HPV 16, 18 and 45 was 82.2%. Infection with HR-HPV showed a significant association for SFRP2 (p=0.05).
Methylation of the SOX17 gene was positively associated with lower severity of stages of cervical cancer (p=0.04). The methylation of the SOX17 gene was associated with the presence of well or moderately differentiated tumors (p=0.01). When all genes were considered an association with better differentiation was observed (p=0.05). In addition, there was a significant association between infection by the HPV 16, 18 and 45 and the diagnosis of adenocarcinoma (p=0.01). A borderline association was observed between the methylation of the DKK3 and SOX17 genes and the diagnosis of adenocarcinoma (p=0.07).

**Conclusion**

The methylation of inhibitors WNT signaling pathway and HPV 16, 18 and 45 infections are frequent events during multistep carcinogenesis, however, only was significant association with SFRP2 methylation. SOX17 methylation was be related with lower cervical cancer severity but not with HPV types. Adenocarcinomas showed a significant association with HPV 16, 18 and 45 infections, however showed a borderline association with DKK3 and SOX17 methylation.
AN MRNA PANEL FOR TRIAGE OF HPV POSITIVE WOMEN WITH HIGH SPECIFICITY FOR DETECTION OF CLINICALLY RELEVANT CERVICAL DISEASE

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Background / Objectives

HR-HPV based primary cervical screening modalities are now being implemented in several countries. Robust objective triage strategies for management of HR-HPV positive women are needed. The objective of this study was to identify mRNA levels of chemokines in liquid based cytology samples for use as biomarkers for the risk stratification of HR-HPV positive women.

Methods

A panel of cervical liquid based cytology samples derived from both screening and colposcopy populations were tested for HR-HPV using the rT-HPV Test (Abbott, USA) and mRNA expression levels of eight chemokines CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL8, CXCL10 and CXCL12 through singleplex TaqMan RT-PCR. A case-control analysis comparing samples from HR-HPV positive women with CIN2+ (n=48) to women with no disease (defined as normal colposcopy or histology <= CIN1, n=80) was performed with ROC curve analysis in order to determine initial clinical performance of the chemokine markers.

Results

Significant differences (p≤0.05) were seen in expression of CCL2 and CCL5 between women with and without significant disease. AUC of CCL2 is 0.61 (95% CI- 0.51-0.71) and CCL5 is 0.60 (95% CI– 0.49- 0.71).

Conclusion

Our initial data show that assessment of chemokine mRNA levels for the detection of HPV associated significant disease has promise. Future work will be aimed at further development and optimisation including the generation of a multiplex real-time PCR which will incorporate the most informative targets.
WHAT IS THE POSITIVE PREDICTIVE VALUE OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL) ON CYTOLOGY FOR THE HISTOLOGICAL DIAGNOSIS OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2 (CIN2) OR MORE? A SYSTEMATIC REVIEW

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Background / Objectives

As cervical cancer is a major health problem, regular cervical screening to make an early diagnosis can help prevent cervical cancer, through identifying and treating pre-invasive cervical lesions. The aim of this review is to evaluate the correlation between the cytological screening and histological outcome in the diagnosis of cervical cancer, more specifically the correlation between HSIL on cytology and histological CIN2+. Learning if cytology brings up information about the probability to discover a high grade cervical intraepithelial neoplasia, would imply that the cytological screening program is a valuable tool on its own.

Methods

An electronic search was carried out in Medline (through Pubmed) and Cochrane (last searched in November 2016), supplemented with the related article feature in Pubmed and snowballing. Article selection (predefined in- and exclusion criteria), data extraction and methodological quality assessment (QUADAS) were evaluated by two independent reviewers.

Results

After identifying 1065 articles, 24 articles were included in this systematic review, representing 51,962 cytological HSIL women in total. The mean CIN2+ percentage in cytological HSIL women is 65,1% (range: 45,4% – 95,2%). The mean CIN3+ percentage in cytological HSIL women is 43,9% (range: 36,4% – 62,1%).

Conclusion

In this systematic review, the mean CIN2+ percentage in cytological HSIL women is 65,1%. The correlation between HSIL on cytology and histological CIN2+ is therefore fair but a biopsy is necessary to confirm high-grade disease.
P13-02
Comparison of HPV positivity of vaginal samples harvested by gynecologist and patient herself in Japan

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Background / Objectives

It seems to be difficult for young Japanese girls to seek gynecological clinic and mount on the examining table because of embarrassment. In order to increase rate of cervical cancer examining test in young girls, it may reasonable to adopt HPV self-harvesting test (S sample). However, before adopting self-harvesting test, it is necessary to verify the superiority or non-inferiority of that compared to harvesting by a gynecologist (G sample). In this report, the superiority of self-harvesting test was examined.

Methods

Seventy four patients having more than slight dysplasia of the cervical region were examined in this study by using paired samples taken by a gynecologist and a patient herself in the same visiting date. Samples were examined by using a kit of GENOSEARCH HPV 31 which can identify 31 HPV genotypes including 13 high risk and 18 low risk HPV types.

Results

In a result, HPV positive rate was higher but not significant in S samples of 78.4% (58/74 cases) compared to that in G samples of 70.3% (52/74 cases). Superinfection of HPV was likely to be detected more often in S samples than G samples. In each type of high risk HPV, positive case number of S samples was superior to that of G samples in most HPV types (7 vs 1). Quite same was observed in the case of low risk HPV (7 vs 1).

Conclusion

In summary, the HPV positivity of S samples was superior to that of G samples. It is considered that HPV sample-harvesting by patient herself is very useful not only for early diagnosis but also for early treatment of cervical cancer.

References

DISCORDANT RESULTS BETWEEN ONCOGENIC HUMAN PAPILLOMAVIRUS RNA AND DNA TESTS IN A COTESTING CERVICAL CANCER SCREENING PROGRAM.

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Background / Objectives

The aim of this study is to evaluate the discordant results between Cobas 4800 HPV test and E6/E7 mRNA Aptima HPV Assay.

Methods

We have studied 736 cervical samples, which were obtained from women attending gynecology practitioners, in the cervical pathology unit from our hospital, in a routine cervical cancer screening program.

All specimens were collected with PreservCyt transport medium.

Each sample was analyzed with Cobas 4800 HPV (Roche Molecular System, Inc.), E6/E7 mRNA-based Aptima® HPV (AHPV; Hologic, Inc) and the discordant results between them, were analyzed by Linear Array HPV Genotyping test (Roche Molecular System Inc).

In each patient we made Pap smears, and biopsy and p16 when the patient required it.

Statistics analyses was done with SPSS 18 for windows.

Results

The average age was 38.02 (19-90).

The prevalence of HPV in each test is shown in Table 1

<table>
<thead>
<tr>
<th>HPV</th>
<th>DNA Cobas 4800</th>
<th>mRNA AHPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>50.1% (369) p=0.032</td>
<td>41.0% (302)</td>
</tr>
<tr>
<td>Negative</td>
<td>49.9% (367)</td>
<td>59.0% (434)</td>
</tr>
</tbody>
</table>
Kappa value DNA Cobas 4800 = 0.834 ; Kappa value mRNA AHPV = 0.805

We calculated the sensitivity and specificity for both techniques:

mRNA E6/E7 AHPV sensitivity = 0.83 [95% CI: 0.79-0.87] specificity = 0.99
[95% CI: 0.94-0.99]

Cobas 4800 DNA sensitivity = 0.94 [95% CI: 0.91-0.96] specificity = 0.90
[0.95% CI: 86-0.92]

Our Pap smear distribution, and the frequency of HPV in each category is shown in Table 2

<table>
<thead>
<tr>
<th>Cytology</th>
<th>% Cobas 4800 Positive</th>
<th>% APTIMA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=450)</td>
<td>28.4</td>
<td>20.4</td>
</tr>
<tr>
<td>ASCUS (n=56)</td>
<td>75.0</td>
<td>62.5</td>
</tr>
<tr>
<td>LSIL (n=108)</td>
<td>85.1</td>
<td>74.1</td>
</tr>
<tr>
<td>HSIL (n=112)</td>
<td>91.9</td>
<td>84.0</td>
</tr>
</tbody>
</table>

10 samples were AHPV positive/Cobas 4800 Negative. Eight of them were negative in the Linear Array Genotyping test. Regarding the two remaining samples, one was positive for HPVs 42+51 and the other was positive for HPVs 16+35+42+51.

74 samples were AHPV negative/Cobas 4800 positive:

- 2 cases out of 74 were negative in Linear Array Genotyping test.

- In the remaining 72 cases: HPV 16 was present in 27% of these cases, HPV 18: 1.7%, HPV 31: 5.4%, HPV 33: 2.7%. All the remaining samples were positives for other HPV genitopes.

50% of them were coinfected with two or more viruses.

Conclusion

According to our data, the sensitivity of Cobas 4800 HPV test was higher (p=0.032).

Almost 30% of the samples with discordant result, E6/E7 mRNA-based Aptima HPV negative and Cobas 4800 HPV positive, had HPV 16/18.

In the screening programs of general population, we need to get more data, as E6/E7 mRNA-based Aptima HPV we are going to loose women with high risk HPV 16/18.
References

UPGRADING OF INFORMATION SYSTEM FOR MANAGEMENT AND MONITORING OF SLOVENIAN CERVICAL CANCER SCREENING PROGRAMME

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Background / Objectives

Due to rapid technology development and research, new evidence that accumulates and will continue to accumulate, enables risk-stratified screening and management of women participating in organised screening programmes. However, it is highly impractical for clinicians to integrate all this knowledge into classical, diagram based clinical algorithms that have traditionally guided clinical decisions. With the aim to overcome the complexity of clinical decisions we have decided to develop a concept of an innovative information system that would in the future enable cervical cancer screening and management of screen-positive women according to their risk for high-grade cervical lesions and cervical cancer within the organised, population based cervical cancer screening program in Slovenia.

Methods

Literature review was done to identify the most relevant information for stratifying individual risk for high-grade cervical lesion in such a way that screening, diagnostic and follow-up algorithms could be adjusted. Innovative IT supported solutions for clinical management decisions and guidance were identified in collaboration with IT experts.

Results

The following risk-stratifying factors were considered for the screening and management: women’s age, HPV-vaccination status, screening history, screening and follow-up tests and their results, colposcopy and histology report. The concept of upgraded central cervical cancer screening information system was developed with the objective that this information will be available to the professionals involved in screening and management of the women, together with the guidance tool for the clinical decisions based on current screening and management guidelines of Slovenian cervical cancer screening program. The system is based on structured, standardised reports and process platforms. New evidence can lead to a change in screening and management guidelines. Due to high information system flexibility the changes will be implemented only by parametrisation and configuration of the system without major changes in programming code.

Conclusion
Organised, population based screening programmes are entering the era where innovative technology solutions and new evidence from research are accumulating rapidly. This may change the traditional role of cancer screening registries from being used as an additional system within the screening programmes that allows for monitoring and evaluation of the programme, to the central communication and decision supporting tool between the professionals involved in screening, diagnostic, follow-up and treatment of women. Such active system also enables real-time monitoring and evaluation of the program.
Economic analysis of a strategy to improve cervical cancer screening in Denmark: Cytology with HPV triage vs. primary HPV screening with cytology and CINtec PLUS cytology triage

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Background / Objectives

Healthcare decision makers search for cervical cancer (CxCa) screening strategies that produce better clinical outcomes while controlling the cost. Possibilities for the introduction of primary HPV screening are currently investigated in Denmark. This modelling study compares clinical outcomes and costs of replacing; 1) cytology with pooled HPV triage (current practice), with 2) cobas® HPV with cytology and CINtec PLUS Cytology® triage (comparator) in the national CxCa screening programme in Denmark.

Sensitivity limitations and subjective interpretation of cytology may lead to missed diagnosis. The combination of primary HPV screening with cytology and CINtec PLUS cytology triage address this shortcoming. CINtec PLUS cytology confirms a transforming HPV infection by detecting cervical cells where HPV has disrupted cellular control (p16/Ki67+) and predicts which women most likely have precancerous cervical lesions and therefore benefit from an immediate colposcopy.

Methods

The model compares screening performance, clinical outcomes and costs. Screening of a hypothetical cohort of 796,000 Danish 30-59-year-old women and natural progression/regression of the disease are modelled for two screening cycles. In the current practice; women with normal cytology return to routine screening (30-49-year-old in 3 years and 50-59-year-old in 5 years), ASCUS and LSIL results have a reflex HPV triage, and HSILs undergo a colposcopy. In the comparator; HPV-negative women return to routine screening in 5 years. HPV+ are first triaged with cytology. Women with ASCUS or LSIL results have a reflex CINtec PLUS triage. Negative CINtec results and normal cytologies are followed up with an HPV retest and a reflex CINtec PLUS triage in two years. CINtec positives and HSIL cytologies undergo a colposcopy. Test sensitivity and specificity data are from ATHENA trial. Other inputs include the local prevalence of HPV, HPV genotypes 16/18, CIN1-3 and CxCa. All costs are calculated from healthcare provider’s perspective.

Results
The comparator strategy increases the sensitivity CIN2+ from the current 52.1% to 71.1% and maintains the specificity CIN2+ (current 99.1%, comparator 99.3%). The better screening performance reduces annual incidence of CxCa in the screened population from the current 23.0 to 18.9 per 100,000, and annual CxCa mortality from 5.1 to 4.1 per 100,000. The annual cost increase by 6%, from 79.2 to 83.8 million DKK, which is mainly caused by increasing treatment costs.

**Conclusion**

The results suggest that replacing the current practice, with primary HPV screening with cytology and CINtec PLUS triage produces better clinical outcomes and increases costs slightly.
ROLE OF HPV VIRAL LOADS IN GUIDING BIOPSY UNDER COLPOSCOPY FOR ASC-US AND HPV POSITIVE WOMEN

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Background / Objectives

Improvement remains on subsequent management of women with concurrent atypical squamous cells of undetermined significance (ASC-US) cytology and positive HPV results. The aim of our study was to explore the role of HPV viral loads in guiding biopsy under colposcopy for ASC-US and HPV positive women.

Methods

We performed a pooled analysis of 17 population-based cross-sectional studies conducted in China from 1999 to 2008. 30,371 women were screened with liquid-based cytology (LBC), HPV testing (hybrid capture 2, HC2) and visual inspection with acetic acid test (VIA) and diagnosed by colposcopically-directed biopsies. HPV viral loads were stratified as low [1.0, 10.0), intermediate [10.0, 100.0) and high [100.0, +∞) in RLU/CO value. Risks of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) among different viral load groups were analysed with linear trend Chi-square test and relative CIN2+ risks among groups were calculated with logistic regression analysis.

Results

908 ASC-US women with positive HPV and complete biopsy results were included in final analysis, among whom, 649, 170 and 89 women were diagnosed as normal, CIN1 and CIN2+, with median value of HPV viral loads as 23.15(4,46,121.91), 85.53(18.73,367.90) and 95.68(18.95, 370.34), respectively. CIN2+ risks increased significantly with elevating of viral load levels (p trend<0.001). Women with intermediate and high viral loads showed at least 67.60(20.52,222.50) times higher CIN2+ risk than ASC-US but HPV negative women. Among 37 CIN2+ cases missed by colposcopy, 72.9% were at intermediate to high viral load range and this proportion achieved 81.8% among cervical intraepithelial neoplasia grade 3 or worse (CIN3+) cases. As for ASC-US women at low viral load and relative lower CIN2+ risk, though an abnormal colposcopy result did not increase the CIN2+ risk...
significantly, it showed 7.61(1.36, 42.62) times higher CIN3+ risk than a negative colposcopy result.

Conclusion

Intermediate to high HPV viral loads in ASC-US and HPV positive women effectively predict a significantly increased risk of existing CIN2+ and should be biopsied regardless of colposcopy results. As to those with low HPV viral loads, only abnormal colposcopy result are supposed to be referred to guide biopsy, ensuring high CIN2+ detection rate and less biopsy harms concurrently.
P13-07

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1Gynecology Department Hospital San Jorge Huesca (Spain), 2Department of Pathology*, Hospital San Jorge Huesca (Spain)

Background / Objectives
To investigate the efficacy and efficiency of different screening programs along 3 periods of time:

2010-2013: Cytology (conventional or liquid based). HPV in ASCUS.


2016: Primary HRHPV testing. HPV parcial genotyping 16-18 and reflex testing

Methods
The base population in our area is 54,372 women. Trained midwives take screening samples in primary healthcare facilities.

In conventional practice, a referral for colposcopy is based on a cytology result : ASCUS and HPV(+), L-SIL, H-SIL, AGC, HPV 16-18 and negative cytologies at women aged > 30 years, and HPV (+) no 16-18 + 2 consecutive negative cytology results for 2 consecutive years.

HPV determination is performed with Roche cobas® 4800 HPV Test (COBAS).

We assessed CIN outcomes following reflex cytology and HPV genotyping for colposcopy triage.

Results
19893 cytologies (4.72 % pathological) were performed during the period of 2010-2013. The frequency of abnormal results was the following: ASCUS (3.63%), L-SIL (0.91%), AGC (0.025%) and H-SIL (0.15%).

For the 2014-2015 period, 10019 cytologies were performed (4.33% pathological), ASCUS (3.17%), ASC-H (0.08%), L-SIL (0.84%) and H-SIL (0.24%) respectively.
In 2016, 1,565 cytologies were performed (13.23% pathological) ASCUS (9.90%), ASC-H (0.45%), L-SIL (2, 43%) and H-SIL (0.45%)

Over the 2010-2013 periods, 2204 HPV determinations were performed, from which positive results were obtained in 27.08% of the cases. During 2014-2015, 8494 HPV determinations were reported, with 12.05% of the results being positive. 2758 studies were performed in 2016, and 10.88% were positive.

745 biopsies were performed during the period 2010-2016. Between 2010-2013, 233 biopsies were performed, (45.37% positive), 58 of them H-SIL. Over 2014-2015 314 biopsies were performed (50,48% positive), 108 H-SIL.

Lastly, in 2016, 198 biopsies were performed (52,33% positive), 69 H-SIL. If we compare the first and the last screening period, positive biopsies increased 241% and H-SIL diagnosis around 375%.

Conclusion

Comparing the first and the last screening period, cytologies have been reduced in a 68.5%, which means a medical cost saving. A big increment is shown in abnormal cytologies during the HPV primary screening.

Closer follow-up of clinical guidelines explains variations in HPV positivity

No benefits are reported from using co-test as first option for the screening program.

A significant increase in the number of colposcopy biopsies was observed over time, with a slight increase in positive biopsy result, but a better diagnosis of H-SIL.

To develop new screening strategy options with the goal of minimizing unnecessary follow-up visits.

References

P13-08
THE IMPLEMENTATION OF HPV BASED SCREENING IN AUSTRALIA: SUSTAINABLE WORKFORCE IMPLICATIONS.

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Background / Objectives

In December 2017 the National Cervical Screening Programme (NCSP) in Australia will change from a 2 yearly conventional cytology based approach to 5 yearly HPV DNA testing. A positive partial genotyping test result for HPV 16/18 will go to reflex LBC and patient referral for colposcopy; detection of other oncogenic types will undergo reflex LBC triage. Among the myriad technical hurdles that must be cleared and human resource elements affected by this change will be a substantial reduction in the workload of cytology laboratories and the role of cytologists and pathologists. The paradigm shift in the primary screening platform has changed the role of the cytologist to a diagnostic one. Thus the experience of the cytologist in partnership with the pathologist is key to the success of the reflex and co testing follow-up investigations. It is estimated that following the changeover around 1 in 5 cytologists will be retained to service the predicted LBC workload. The immediate challenge of the reshaping period for laboratories that specialise in gynaecological cytology has been to manage the opposing forces of maintaining service efficiencies while their workforce is restructured. Specific planning for the preparation of future cytologists is a challenge facing pathology laboratories, tertiary education centres that train undergraduate scientists in diagnostic cytology and professional bodies responsible for continuing education, quality assurance and performance measures.

Methods

This study aims to encapsulate the available information around the introduction and management of HPV based screening in Australia in 2017 and strategies to deal with pathology workforce issues. Estimates of the changes to the national workload indicate that cytology tests will be considerably fewer (1). Available information around anticipated changes to work practices by major public and private pathology providers in relation to the workforce transition is presented. Strategies that facilitate future diagnostic cytology training at university level and in the workplace are tabled.

Conclusion

The theoretical endpoint for the NCSP in Australia is prevention of all HPV related anogenital carcinoma through vaccination and improved screening outcomes. The role of cytology in this pursuit is changing but will remain a key component for the foreseeable future. Appropriate training regimens for cytologists and pathologists that will ensure ongoing diagnostic acuity are essential for service provision.
References

IS A THREE-YEAR CYTOLOGICAL SCREENING FOR CERVICAL CANCER SAFE AND IS IT IMPORTANT FOR POSSIBLE EARLY TREATMENT OF PREMALIGNANT INTRAEPITHELIAL LESIONS?

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1Gynecologist (Serbia), 2Neurologyst (Serbia), 3Gynecologist (Serbia), 4Oncologist (Serbia)

Background / Objectives

Screening for cervical cancer implies early diagnostics of high-risk intraepithelial lesions (HSIL) and the initial microinvasive changes. The method which is recognized as the gold standard for this screening is exfoliative cytology - PAP test. Recommendations for safe time interval that should repeat cytology screening are 3 years. In this interval, calculated risk of carcinoma is 0,8%. It is known that the sensitivity of conventional PAP test is from 30 - 85%. The influence of subjective factors and errors in collecting and interpreting the findings reduce the validity of the test. According to official recommendations, it is considered that the repetition of PAP test in the three-year interval is safe in screening of cervical carcinoma. The aim of this study was to determine the reliability of conventional PAP test in screening for cervical cancer and to determine what percentage of the HPV test can increase the reliability of PAP test in screening for cervical cancer.

Methods

The study entered 39 patients, which occurred in 2017, for surgical treatment of HSIL and invasive cancer (IC). In all patients, the PAP test was done and also HPV PCR test. A history of time interval since the last normal NILM findings as well as data on the possible contact or irregular bleeding, are also entered in the statistical analysis of data.

Results

Only 20,51% of patients had a PAP test done before three years or more. 35,8% of the patients had normal test result less than 3 years and 43,58% of them done the PAP test a year ago and the result was also normal - NILM. From the 20 patients with IC, 16 of them (80%) had normal PAP test less than 3 years. 40% (8 patients) had a normal PAP test a year ago. Analysis of the documents that they have been brought with them, as part of preoperative preparation, showed in 11 patients (28,2%) with HSIL and IC normal PAP findings. From these 11 false-negative cytological findings (FNF), in 4 (36,36%) has been diagnosed HSIL and in 7 (63,6%) IC. From these 7 with IC, in 4 patients with (57%) FNF, histological type was adenocarcinoma. A total sensitivity of cytology was 71.79%. HPV testing in 38 patients (97%) with HSIL and IC was positive. Only one patients with HSIL (2.56%) had a negative HPV test. HPV test increased sensitivity of cytology for 25.64%.
Conclusion

The three year cytological screening with conventional PAP test, as a alone test, is not reliable for screening of cervical cancer. HPV test, in combination with cytology, increases the overall sensitivity of cytology for 25.64%. Both of them are very important for early treatment of premalignant intraepithelial lesions.

References


P13-10
Compare Two Different Usages of the FRD™ for Detecting High Grade Cervical Lesions and Invasive Cancer

D. Li
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Background / Objectives

To evaluate the two usages of the Folate Receptor-Mediated Staining Solution (FRD™) for detecting CIN2+, and compare it to TCT and HPV testing.

Methods

The FRD™ is a staining method for rapid visualization of CIN2+. Test results are determined immediately with the aid of the FRD™ Colorimeter after staining of the entire cervical epithelia. The two methods of the FRD™ test were performed on patients who had ASC-US & above TCT, and/or positive HPV test, before undergoing colposcopy and biopsy. The first FRD™ testing method (sampling method), the cervical epithelium is collected with the Epithelium Staining Applicator, and then the applicator is stained by the FRD™ staining solution. The second method (direct dyeing method), the Epithelium Staining Applicator is dipped into the FRD™ staining solution, and then the cervical epithelium is collected and stained by the applicator.

Results

317 women with histological findings were included. CIN2+ was found in 109 women (34.38%) including 16 cervical cancer cases (3.3%). CIN1 and negative cases accounted for 9.46% and 56.15%, respectively. TCT results included NILM in 103 women (32.49%), ASC-US in 130(41.01%), LSIL in 51(16.09%), ASC-H in 12(4.73%), and HSIL & above in 21(6.62%). HPV positive rate was 90.54% (287/317). Positive FRD™ test was determined in 35.33% women (112/317) by the sampling method, and 48.90% (155/317) by the direct dyeing method. The sensitivity to detect CIN2+ for abnormal TCT, positive HPV, and positive FRD™ by the sampling method and direct dyeing method were 69.72%, 97.25%, 64.22%, and 81.65%, respectively. The specificity to detect CIN2+ for abnormal TCT, positive HPV, and positive FRD™ by the sampling and direct dyeing method were 37.98%, 12.98%, 78.81%, and 68.27%, respectively.

Conclusion

Compared with TCT and HPV test, both the usages of the FRD™ had a compatible sensitivity and high specificity to detect high grade cervical lesions. The sensitivity of the direct dyeing method was higher than the sampling method, and its specificity was lower than sampling method, but there was no significant difference between them. Sensitivity is more significant in cervical cancer detection, therefore the direct dyeing method of the FRD™ is more suitable in clinical settings. In addition, the FRD™ is a very inexpensive and easy method, which can be used in less-developed
counties or areas that lack the resources and trained personnel required for routine cervical cancer detection.
The Significance of the Epithelium Staining Applicator in Cervical Staining with the FRD™

D. Li
Shaanxi Waiyuan Biomedical Research Institute Co., Ltd. (China)

Background / Objectives

This study was aimed to evaluate the significance of the Epithelium Staining Applicator in cervical staining with the Folate Receptor-Mediated Staining Solution (FRD™) in detecting cervical abnormal lesions (CIN2+), based on biopsy being used as the gold standard.

Methods

The FRD™ test was performed before colposcopy, on patients with abnormal TCT and/or positive HPV test. The cervical epithelium was stained by the Epithelium Staining Applicator, by first dipping the applicator into the FRD™ staining solution, and then by pressing the applicator against the cervix with the aid of speculum. After staining, the Epithelium Staining Applicator was placed into the FRD™ Colorimeter, which scans the applicator for any color change and prints out the scanning results. The FRD™ test results were determined by the readings found on the scanning results.

Results

261 women with histological findings were included in the study. CIN2+ was found in 97 patients (37.16%) including 12 cervical cancers cases (4.60%). CIN1 accounted for 10.34%. TCT results included NILM in 82 women (31.42%), ASC-US in 114 women (43.68%), LSIL in 42 women (16.09%), ASC-H in 7 women (2.68%), HSIL and above in 16 women (6.13%). The HPV positive rate was 87.36% (228/261). A positive FRD™ test was determined in 49.81% women (130/261). The sensitivity to detect CIN2+ lesions for abnormal TCT, positive HPV, and positive FRD™ were 76.29%, 93.81%, and 80.41%, respectively. The specificity to detect CIN2+ lesions for abnormal TCT, positive HPV, and positive FRD™ were 35.98%, 16.46%, and 68.29%, respectively.

Conclusion

The FRD™ is an alternative method which is suitable for cervical cancer detection. In addition, the Epithelium Staining Applicator is a suitable assistive device to complete the FRD™ test.
The diagnostic value of lugol solution, acetic acid, and Pap smear compared to biopsy regarding premalignant and malignant cervical lesions diagnosis in patients in need of colposcopy

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Background / Objectives

Given the high incidence of cervical cancer in developing countries and the importance of prompt diagnosis and treatment regarding mortality reduction, developing accurate and cost-effective method for screening and diagnosis of this cancer has occupied the minds of physicians for years. Previous studies reported high diagnostic accuracy for Pap smear, VIA, and VILI with respect to cervical cancer. Moreover, not all developing countries have access to colposcopy. Therefore, this study was attempted to compare these three tests with colposcopy in terms of diagnostic value.

Methods

This diagnostic study was conducted on 328 women referred to Shahid Sadoughi Clinic for colposcopy. At the first step, Pap smear was performed for those who did not undergo this test previously. Then, all the participants underwent VIA and VILI tests according to the known protocol. Next, colposcopy was conducted for all the participants, biopsy sample was obtained, and histological features were studied. Finally, the results were compared based on statistical indicators.

Results

Sensitivity of 91.9% and specificity of 53.6% were obtained when Pap + VILI + VIL test results were compared with biopsy.

Positive predictive value of 20% and negative predictive value of 98% achieved.

A diagnostic accuracy of 58% was gained in case of positive CIN II results and higher degrees of CIN, the findings were as follows when colposcopy results were compared with biopsy ones:

Sensitivity of 86.5% and specificity of 95.5% were obtained.
Positive predictive value of 71.1% and negative predictive value of 98.2% were acquired.

A diagnostic accuracy of 94% was attained.

Conclusion

According to our findings, Pap+ VIA+ VILI test results are comparable with colposcopy ones in terms of diagnostic accuracy (73% and 75%, respectively). Therefore, Pap+ VIA+ VILI test is recommended as a competent alternative to colposcopy in developing countries.

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HPV analysis improves the PPV of Atypical Glandular Cells

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Background / Objectives

The finding of atypical glandular cells (AGC) is important for the possible prevention of endocervical adenocarcinoma (ADCA). This diagnosis group is quite unspecific and includes several cases with reactive conditions as well as dysplastic squamous lesions. The purpose of this study is to determine how reflex HPV analysis may improve the positive predictive value (PPV) of AGC.

Methods

During 2014 - 2015, altogether 385 LBC samples (ThinPrep®, Hologic) were diagnosed as AGC. Reflex HPV analysis were performed by the Cobas 4800 platform (Roche Diagnostics). Histological follow-up was available in 206 (54%) cases - 105 (51%) of these containing HR-HPV.

Results

The HPV positive group contains 3 cases of cervical ADCA and 15 cases of adenocarcinoma in situ (AIS) together with 51 cases of high grade squamous intraepithelial lesion (HSIL). The PPV for a lesion to treat was 69/105 (66%). The corresponding figures for the HPV negative group was 3 endometrial carcinomas, 1 metastatic breast carcinoma and 2 HSIL, giving a corresponding PPV of 6/101 (6%). HR-HPV was found in 69/71 cases with cervical lesions to treat (sensitivity 97%).

Conclusion

The results highlight the importance of combined cytology and HPV analysis. HPV defines AGC cases with an exceptionally high PPV for high grade lesion to motivate follow-up.

References


EVALUATION OF A HOST DNA METHYLATION PANEL IN A HIGH HPV PREVALENCE COHORT

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Background / Objectives

Today there is a strong agreement in the scientific community regarding the hr-HPV testing superiority in cervical cancer primary screening versus cytology. However, due to the limited specificity of hr-HPV testing, new biomarkers are needed in order to triage the positive cases, avoiding overtreatment and excessive referral to colposcopy. DNA methylation (viral and host) has been proposed as a promising strategy. This work aims to evaluate clinical specificity and sensitivity of a host methylation panel proposed by Hansel et al., in 2014 with some modifications (GynTect®).

Methods

A maximum of 100 consecutive PreservCyt® samples diagnosed with LSIL and histology follow-up were selected from the routine diagnostics at LAP Unilabs Porto. In order to access sensitivity some other samples with histologically confirmed CIN3+ lesions were also included. All results were compared with previous cytology, HPV status, CINtec® Plus results and histology data available for that sample. Information regarding the evaluated sample, patient information and data regarding other clinical history related to that patient were taken from the laboratory database to allow the clinical significance of the results to be assessed. All data stored for the evaluation was anonymized. The gold standard is histologically confirmed cervical intraepithelial neoplasia (CIN) grade 3+. Sensitivity and specificity of each of the triage tests was calculated based on disease defined as CIN 3+ (p16 stain confirmed).

All DNA methylation panel testing were performed in the Roche cobas®480z analyzer (component of the cobas®4800 system).

Results

Data is being collected, but the results are not yet fully available.

Conclusion

Preliminary data confirms the capability of the markers to detect high grade disease, with a low false-positive rate.

References

Biological risk factors associated with methylation positive and negative high-grade cervical lesions – clinical study

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Background / Objectives

Cervical carcinogenesis is a multistep process which starts with an acquisition of high-risk HPV infection. Multiple biological and behavioural risk factors affect the progression towards severe lesions. The most pronounced risk factors include viral factors, immunosuppression, co-occurrence of other sexually transmitted infections (STI), genetic polymorphism of tumour suppressor genes (TSG), and smoking. Risk factors contribute to the transition from incident to permanent HPV infection and from persistent infection to high-grade lesion.

HPV-induced methylation silencing of TSGs is believed to be a sign of high-grade lesions carrying higher risk of short-term progression into invasive stadium. While almost all cases of invasive carcinomas have methylated promoters of specific TSGs, less advanced severe cervical lesions (HSIL) are methylated only in 60-90 % cases. Morphology or biomarkers reflecting differences between methylated and unmethylated HSIL lesions are scarcely described in the literature.

In our study, we focused on exploring biological risk factors in methylated and unmethylated HSIL lesions, namely co-occurrence of other STIs and presence of certain HR-HPV genotypes.

Methods

108 residual samples of liquid-based cytology of Czech women with histologically confirmed high-grade cervical lesion were analysed with

1) Precursor M kit (Self-screen) to assess methylation status of tumour-suppressor genes CADM1, MAL, and has-124, related to cervical carcinogenesis

2) LINEAR ARRAY HPV Genotyping Test (Roche) and type-specific PCR targeting E6 and E7 viral oncogenes to reveal specific HR-HPV genotypes
3) Allplex STI Essential Assay (SeeGene) to discover possible co-occurrence of 7 most common microbial pathogens responsible for cervicitis

Methylated and unmethylated HSILs were evaluated in two categories, first presence of HPV genotypes 16,18 and 45, and second co-occurrence of any microbial pathogen.

Results
36% of HSIL lesions in our study had negative methylation status. At least one microbial pathogen was detected in 50% of HSIL lesions but there was no significant difference between methylated and unmethylated groups. HPV types 16, 18, and 45 were detected more often among methylation positive samples but this finding was not statistically significant.

Conclusion

There is no difference in STIs co-occurrence between methylation negative and positive HSILs. HPV types 16, 18, and 45 occur more often in methylation positive HSILs but also infection of other HR-HPV types might result in methylation of TSG’s promoters. Further studies are needed to confirm that the methylation status differentiates biological early lesions from advanced HSILs.

References

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THE INTERACTION BETWEEN HPV INFECTION AND BACTERIAL MICROBIOTA IN PLACENTA, CERVIX AND ORAL MUCOSA

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Background / Objectives

Objective. We aimed to investigate whether an existing HPV infection has influence on the bacterial microbiota composition in the placenta and cervix as well as in the maternal and infant oral mucosa.

Methods

Materials and methods. This study is a nested case-control study based on samples collected in the prospective Finnish Family HPV Study. Total 39 families were selected for this study based on placenta HPV status, the mode of birth and availability of samples (13 cases with HPV positive placenta and 26 controls with HPV negative placenta of which 13 were obtained through vaginal delivery and 13 by Caesarean section). The corresponding maternal cervical and oral and infant oral samples were selected for analyses.

HPV DNA genotyping of 24 different genotypes (6 low-risk and 18 high-risk types) of the samples was conducted using Multimetrix® assay (Multimetrix, Regensburg, Germany). Microbiota composition and diversity was characterized by 16S rRNA gene sequencing (V1-V3 region, Illumina protocol, Illumina, San Diego, CA, USA).

Results

Results. HPV DNA was found in 23% (9/39) maternal cervix, 33% (13/39) maternal oral, and 45% (18/40, included one set of twins) infant oral samples. HPV16 was the most frequent type found in all groups studied (54% of placenta, 22% of cervix, 54% of maternal oral and 39% of infant oral samples).

In maternal mouth, HPV positive samples displayed significantly higher richness (Chao1 index) of bacterial microbiota (p=0.032) but no difference in Shannon index. HPV status did not influence microbial diversity and richness in the other samples.
The HPV positive cervix harboured significantly more *Adlecreutzia* (*p*=0.048), *Mycoplasma* (*p*=0.048) and *Gemella* (*p*=0.0058) genus as compared to HPV negative cervical samples. In maternal oral samples, *Selenomonas* spp. was significantly increased (*p*=0.012) in HPV positive individuals whereas the amount of *Propionibacterium* (*p*=0.026) and *Staphylococcus* (*p*=0.049) were increased in HPV positive infant oral samples. In the placenta, *Lactobacillus* (*p*=0.076) were slightly increased in HPV positive samples compared to placenta HPV negative.

**Conclusion**

**Conclusion.** HPV infection is associated with altered bacterial microbiota composition in the placenta and mouth. Whether the changes in bacterial microbiota predispose or result from HPV remains to be determined in future studies.
Background / Objectives

The bacteria in the human vagina have an important role in maintaining general health and protecting host from pathogenic microbes. Our knowledge about vaginal microbiota and its complexity has expanded vastly after development of novel culture-independent methods. Yet the big picture of vaginal microbiota remains the same as when Döderlein first found Lactobacillus from vagina.

In recent studies, the human papilloma virus (HPV) infection and its clearance rate been linked with vaginal microbiota type and bacterial vaginosis (BV) [1,2]. This emphasizes the need for better understanding of the function of different microbiota types and their interplay with the host.

Methods

We sampled 50 healthy Finnish women during routine Pap smear screening for cervical cancer in Helsinki, Finland. We collected an extensive background questionnaire and swabs for microbiota and HPV analysis. The Pap smears were reanalyzed to classify microbiota features visible to microscope. For bacterial community profiling, we used Illumina HiSeq platform to sequence hypervariable V3-4 regions of the 16S rRNA gene. For estimation of different strains among observed species we used minimum entropy decomposition (MED) [3] and oligotyping [4] and for functional analysis we used PICRUST [5] and other similar methods.

Conclusion

We have just started to analyze the data. The preliminary analysis identified interesting associations between the microbiota, socioeconomic factors and on the other hand between the Pap smear microscopy and sequencing. The results and conclusions will be presented at the conference.

References


DEVELOPMENT AND VALIDATION OF AN OPTIMIZED HPV COMPETITIVE LUMINEX IMMUNOASSAY (9-PLEX) AND HPV IgG ANTIBODY DETECTION LUMINEX IMMUNOASSAY (9-PLEX) SUPPORTING CLINICAL SEROLOGY TESTING FOR GARDASIL-9

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Background / Objectives

Two multiplex Luminex immunoassays are used to assess antibody responses in MSD Gardasil-9 clinical trials: (1) The primary immunoassay is the HPV 6, 11, 16, 18, 31, 33, 45, 52, 58 competitive Luminex immunoassay (HPV-9 cLIA) and (2) the secondary assay, the HPV 6, 11, 16, 18, 31, 33, 45, 52, 58 total IgG Luminex immunoassay (HPV-9 IgG assay), is used for supportive analyses. Recently, both assays were re-developed, and the optimized assays were validated and approved by the Center for Biologics Evaluation and Research, U.S. Food and Drug Administration. In addition, the assays were formally bridged to the previous assay versions to assess serostatus cutoffs (SSCO) and the impact of the changes on persistence studies.

Methods

The optimization of the assays included assessment of the following parameters: VLP coating concentration, wash buffer, Luminex microspheres, serum sample and reference standard diluent, reference standard starting dilution and titration series, and vendor and concentration of the PE-labeled antibodies. For both assays, the validation studies evaluated various performance parameters including intra-assay precision (repeatability), intermediate precision, linearity, relative accuracy/dilutability, and limits of quantitation. For the bridging study, individual patient sera from an MSD clinical trial, including day 1, month 7, and month 36 serum samples from 100 subjects, and an additional 50 day 1 samples, were used to compare measured concentration results to the historical values.

Results

Analysis of the validation data indicates that the optimized HPV-9 cLIA and IgG assays are accurate, specific, and precise throughout the quantifiable range for each HPV type. Results of the bridging study indicate that there is a strong positive linear
association between the assay versions. For both HPV-9 cLIA and IgG assays, the SSCOs were adjusted to align seropositivity rates between assay versions.

Conclusion

Optimization of the assay, including the elimination of antibody-depleted human serum in the assay buffer and increasing the starting dilution from 1:4 to 1:10, led to an improvement in the dilutability of the HPV-9 cLIA (within 1.25-fold per 10-fold dilution) relative to the prior version. For both HPV-9 cLIA and IgG assays, the strong positive linear association between the previous version and optimized version allow for immunogenicity assessments of long-term follow-up studies across assay versions.
A NEW GENERATION OF VALIDITY TESTING FOR ONCOPROTEIN-BASED CERVICAL CANCER SCREENING


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Background / Objectives

HPV is known to infect basal keratinocytes found within the cervical transformation zone. Promising new HPV tests are based on the detection of viral oncoproteins of hrHPV types, but their diagnostic capabilities may be limited without a way to assess specimen validity. Hence, there is a need to reduce false negative results of these tests due to unreliable sampling. Here we describe a new assay that captures cytokeratins 5, 8 and 18 from potential target cells as a means of normalizing cervical specimens.

Methods

A keratin 5/8/18 sandwich ELISA – recomWell Keratin 5/8/18 - was developed for detection of cells located within or originating from the cervical transformation zone. Content of different cell types was validated microscopically. Suitable for measurement of Keratin are liquid-based cytological samples in PreserveCyte.

Results

The Keratin ELISA was successfully validated with cell lysates of HPV positive and negative cell lines of cervical origin. The proof of concept was shown by measurement of well characterized clinical samples. In 335 HPV positive samples of all stages of CIN, Keratin 5/8/18 could be detected with a similar signal distribution when compared to 1484 normal samples (OD 0.96 +/-0.17). 94.5% of all samples and 96.1% of normal samples showed signals for Keratin 5/8/18 above cut off. On the contrary, 90.4% of samples with CIN2+ and 89.1% with CIN3+ were positive for Keratin 5/8/18.

Conclusion

Our results demonstrate the presence and detectability by ELISA of Keratins 5, 8, and 18 in parabasal, squamous metaplastic, and endocervical cells, while simultaneously suggesting their absence in differentiated squamous cells. We also validated the expression of these Keratins in individuals with HPV-induced dysplasia.
and found differences in the proportion of valid samples between healthy woman and those which developed CIN2+. Furthermore, recommendations for interpretation of the results of the combined test systems (validity testing by recomWell Keratin ELISA and HPV E7 oncoprotein testing by recomWell HPV 16/18/45) were set.

The recomWell Keratin 5/8/18 allows validity testing of cervical samples by detection of potential HPV target cells and could therefore be a means to decrease the rate of false negative HPV results due to unreliable sampling.
P19-02
ESTABLISHMENT OF THREE-DIMENSIONAL ORGANOTYPIC RAFT CULTURES CLOSELY MIMICKING HPV-TRANSFORMED CERVICAL LESIONS IN AN EPITHELIAL CONTEXT

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Background / Objectives

HPV-transformed cancer cell lines have so far been intensively studied and characterized in monolayer cultures. To develop novel medical treatment options a three-dimensional (3D) model of HPV-transformed cells in their natural context is of superior importance when analyzing drug effects on these cells. We intended to create a 3D organotypic epithelial raft culture resembling HPV-induced (pre-)cancerous lesions. This will allow better evaluation and understanding of the effects of novel therapeutic options on HPV-transformed cancer cells in their natural context as well as on surrounding healthy keratinocytes.

Methods

A dermal equivalent comprising a fibrin gel containing human fibroblasts embedded in a tissue scaffold was created in a deep-well plate. A combination of primary human keratinocytes and cervical HPV-transformed cancer cell lines was then seeded on the dermal equivalent and grown to full confluency over the course of 24 hours. Epithelial differentiation of the keratinocytes occurred over the course of 2 weeks. The cultures were subsequently harvested. Histological slices were prepared and stained with hematoxylin & eosin. Furthermore, combined p16INK4a/Ki67 immunohistochemical staining as well as combined Keratin 14/Keratin 7 immunofluorescence were performed.

Results

After two weeks of culture we observed a fully differentiated epithelium comprising healthy keratinocytes and clearly distinguishable lesions consisting of HPV-transformed cancer cells. p16INK4a/Ki67 immunohistochemical staining allowed for a clear distinction between normal keratinocytes and HPV-transformed cells with only HPV-transformed cells staining positive for both markers. Likewise, Keratin 14/Keratin 7 immunofluorescence granted a specific identification of the HPV-transformed cervical cancer cells. These transformed lesions, established with various HPV-transformed cell lines, resemble actual lesions found in a natural environment.

Conclusion
A 3D organotypic raft culture growing HPV-transformed cells in a more natural setting was established, providing an ideal model to study effects of future therapeutic approaches.
P20-01
RETROSPECTIVE STUDY OF SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF CYTOLOGY IN LIQUID MEDIUM, HPV DNA TEST AND GENOTYPING FOR HPV16, IN DIFFERENT CERVICAL CANCER SCREENING SCENES

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Background / Objectives

Estimation of sensitivity (SE), specificity (SP), positive and negative predictive value (PPV/NPV) of cytology in liquid medium, HPV DNA test and partial genotyping for HPV16 in women older and younger than 30 years old in 3 screening periods: 2010-13, 2014-5 and 2016 (Huesca General Hospital, Spain).

Methods

Our target population is 54,372 women. Cytologies are performed in liquid medium. HPV determination is performed by COBAS 4800 Roche platform. From 2010 to 2013, screening was based on triennial cytology and HPV DNA test in ASC-US; during 2014-2015 period on co-testing in > 30 years-old women, and from 2016 on HPV DNA test with reflex cytology and partial genotyping for HPV16-18. The burden of disease is based on colposcopy biopsies.

Results

<table>
<thead>
<tr>
<th>Table 1. Cytology in liquid medium.</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>2010-13 (n=197)</td>
</tr>
<tr>
<td>SE&lt;30 years-old</td>
</tr>
<tr>
<td>SE&gt;30</td>
</tr>
<tr>
<td>SP&lt;30</td>
</tr>
<tr>
<td>SP&gt;30</td>
</tr>
<tr>
<td>PPV&lt;30</td>
</tr>
<tr>
<td>PPV&gt;30</td>
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</table>
### Table 2. HPV DNA test

<table>
<thead>
<tr>
<th></th>
<th>2010-13 (n=62)</th>
<th>2014-15 (n=248)</th>
<th>2016 (n=187)</th>
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<tr>
<td><strong>SE&lt;30 years-old</strong></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>SE&gt;30</strong></td>
<td>100%</td>
<td>98.8%</td>
<td>98.5%</td>
</tr>
<tr>
<td><strong>SP&lt;30</strong></td>
<td>0%</td>
<td>2.7%</td>
<td>21.4%</td>
</tr>
<tr>
<td><strong>SP&gt;30</strong></td>
<td>3.5%</td>
<td>7.9%</td>
<td>14%</td>
</tr>
<tr>
<td><strong>PPV&lt;30</strong></td>
<td>18.8%</td>
<td>32.1%</td>
<td>47.6%</td>
</tr>
<tr>
<td><strong>PPV&gt;30</strong></td>
<td>37.8%</td>
<td>42.9%</td>
<td>46.8%</td>
</tr>
<tr>
<td><strong>NPV&lt;30</strong></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>NPV&gt;30</strong></td>
<td>100%</td>
<td>90%</td>
<td>92.3%</td>
</tr>
</tbody>
</table>

### Table 3. HPV DNA type 16

<table>
<thead>
<tr>
<th></th>
<th>2010-13 (n=29)</th>
<th>2014-15 (n=127)</th>
<th>2016 (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SE&lt;30 years-old</strong></td>
<td>66.7%</td>
<td>64.7%</td>
<td>90%</td>
</tr>
<tr>
<td><strong>SE&gt;30</strong></td>
<td>52.9%</td>
<td>60%</td>
<td>47%</td>
</tr>
<tr>
<td><strong>SP&lt;30</strong></td>
<td>61.5%</td>
<td>64.9%</td>
<td>57.1%</td>
</tr>
<tr>
<td><strong>SP&gt;30</strong></td>
<td>55.2%</td>
<td>51.8%</td>
<td>75.6%</td>
</tr>
<tr>
<td><strong>PPV&lt;30</strong></td>
<td>28.6%</td>
<td>28.6%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>PPV&gt;30</strong></td>
<td>40.9%</td>
<td>46.6%</td>
<td>59.6%</td>
</tr>
<tr>
<td><strong>NPV&lt;30</strong></td>
<td>88.9%</td>
<td>80%</td>
<td>88.9%</td>
</tr>
<tr>
<td><strong>NPV&gt;30</strong></td>
<td>66.7%</td>
<td>64.8%</td>
<td>65%</td>
</tr>
</tbody>
</table>

**Conclusion**

Cytology shows a progressive reduction of sensitivity and specificity in < 30 years-old women. There are also marked variations in specificity and NPV in women > 30. These changes may be due to new screening paradigms and to the incorporation of younger pathologists. On the other hand, PPV increases with the incorporation of HPV to screening.
Sensitivity for HPV DNA test is around 100%, regardless of age and analyzed period. Its specificity has increased with the systematic HPV DNA test (following a standard protocol) and with patient selection following consensus guidelines.

Positivity for HPV16 is associated with a decrease in sensitivity, specially in women > 30 years-old, coupled with a spectacular increase in specificity, mostly in women > 30. Likewise, a moderate increase in PPV is corroborated, with no relevant age differences. In addition, there is a slight to moderate decrease in NPV, regardless of age and screening periods.

The explanation to low specificity of HPV DNA test may be that 2/3 are positive to some of the types NO16-18.

The high sensitivity and low specificity of the HPV DNA test make us incorporate more specific techniques that reduce unnecessary colposcopies. Perhaps dual staining help us to refine derivation scheme.

References

Conization using electrosurgical conization with cold coagulation for cervical intraepithelial neoplasia: a feasible treatment with a low risk of residual disease

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Background / Objectives

Objective: This study was performed to evaluate the significance of positive resection margins (RMs) of electrosurgical conization with cold coagulation as definitive treatments for patients with cervical intraepithelial neoplasia (CIN).

Methods

Methods: We retrospectively reviewed 306 patients who underwent electrosurgical conization with cold coagulation for CIN treatment at our institute from August 2005 to December 2016. A right-angled triangular loop in a single pass followed by a cold coagulator (120°C) to the cone bed for 10 to 20 seconds was used. Patients with positive RMs were underwent pap smear, human papilloma virus (HPV) DNA testing, and endocervical curettage after 3-6 months without additional treatments. Patients with margin positive invasive carcinoma or adenocarcinoma in situ (AIS) recommended hysterectomy, firstly. Pathologic reports and clinical data were obtained and evaluated.

Results

Results: Histopathological evaluation of electrosurgical conization materials revealed the presence of CIN I in 54, CIN II/III in 241, AIS in 3, and invasive carcinoma in 8 (microinvasive/adenocarcinoma, 7/1, respectively) patients. Margins were positive in 41 (13.4%) cases; 0 in CIN I, 37 in CIN II/III (15.4%), 1 in AIS (33.3%), and 3 in invasive carcinoma (37.5%), respectively. Twenty-eight patients had positive endocervical RMs, while thirteen patients had positive exocervical RMs. In this series, there were no cases with simultaneous positive endocervical and exocervical RMs. Six patients with positive margins were lost to follow-up. Two CIN cases with positive RMs revealed 1 CIN I and 1 CIN III at first follow-up. Three microinvasive carcinoma cases revealed 1 no residual disease, 1 CIN I, and 1 CIN II after hysterectomy. However, one adenocarcinoma case without positive RM and one AIS case with positive RM revealed no residual disease after hysterectomy. Totally, four out of 300 patients (1.3%) who underwent electrosurgical conization with cold coagulation had residual diseases.

Conclusion

Conclusions: These results suggest that electrosurgical conization with cold coagulation is
a feasible treatment for CIN cases with a low risk of residual disease. Patients who are diagnosed with CIN preoperatively could be followed up without additional treatments in spite of positive RMs.

References

Key Words: Cervical intraepithelial neoplasia, Microinvasive carcinoma, Electrosurgical conization, Cold coagulation, Resection margin
Evaluation and correlation of primary histopathological diagnosis of targeted biopsy till the final histopathological diagnosis following diagnostic and therapeutic process of leep conization

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Background / Objectives

Evaluation of consistency of the final histopathological diagnosis following diagnostic and therapeutic process of leep conization till the primary histopathological diagnosis following targeted cervical biopsy.

Methods

The analysis included 540 patients, in whom leep conization was performed due to incorrect result following targeted biopsy – SIL or discrepancy of cytological – histopathological results. Targeted biopsy and leep conization were performed by doctors with years of experience. Colposcopy was performed using stereoscopic colposcope (Olympus OCS-500). Colposcopic pictures were evaluated according to Reid’s index, regarding the margin and acetowhiteness, iodine negative test and vascularization.

Results

The most frequent histopathological diagnosis following targeted biopsy and leep conization were HGSIL-CIN 2 type changes, which comprised 36% and 34% respectively and HGSIL-CIN 3 type changes which comprised 21% and 27% respectively. Consistency of histopathological results of targeted biopsy compared to the final result following leep conization comprised 94% for HGSIL type changes.

Conclusion

Targeted cervical biopsy and leep conization show very high consistency in terms of histopathological results.
WHAT’S BEHIND LSILs?


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Background / Objectives

Low grade intraepithelial lesion (LSIL) is the second most common anomaly on cervical cytology. Despite the low risk of progression to carcinoma, 10-25% have a histological diagnosis of cervical intraepithelial neoplasia grade 2 or higher (CIN2+). Therefore, the challenge these lesions present is to identify the patients at risk of developing premalignant or malignant lesions. Our aim was to evaluate demographic characteristics, colposcopic findings and clinical follow-up of patients with LSIL on cervix cytology.

Methods

Retrospective longitudinal study of the patients referred to our Cervical Pathology consultation during January-December 2014 (n=356). Colposcopic classification was performed according to 2011 IFCPC nomenclature and histologic classification was divided in three groups: No displasia, CIN 1 and CIN2+. Statistical analysis was performed using SPSS® v.21.

Results

LSIL was the indication for referral of 36% (n=128) of patients. The mean age at referral was 38.46±10.49 years, 14.1% (n=18) were post-menopausal, 64.6% (n=52) had >1 sexual partners, 20.3% (n=16) were smokers, 63.6% (n=70) were contraceptive pill users and 39.6% (n=40) were positive for high risk HPV. 96.4% (n=124) had a colposcopy done upon admission: 33.9% (n=42) were normal, 62.1% (n=77) had grade 1 findings and 3.9% (n=5) had grade 2 findings. Within those with grade 1 findings, biopsy revealed no displasia in 63.6% (n=49), CIN 1 in 27.2% (n=21) and CIN2+ in 9.1% (n=7). Within the group of grade 2 findings, one case presented no displasia, one case presented a CIN1 and 3 cases presented CIN2+. There was a significant association between grade 2 colposcopic findings and high grade histologic lesions (OR 13.714; IC 1.978-98.065). Regarding the therapeutic approach, CIN1 lesions underwent expectant management in 63.6% (n=14) and destructive therapy in 36.4% (n=8). All CIN2+ lesions were submitted to excisional therapy. During a 6-24 month follow-up period, there were no de novo high grade lesions. There was no case of cervical cancer in our sample.

Conclusion

In accordance with the literature, LSILs were more prevalent in premenopausal women, with a higher number of sexual partners and a high prevalence of high risk HPV. Despite traducing mostly low grade histologic lesions, CIN2+ was present in
12.3%. The presence of grade 2 colposcopic anomalies correlated with high grade histologic lesions, reinforcing the importance of colposcopy in the surveillance of these patients.
P20-05
CYTOLOGICAL CHANGES IN WOMEN UNDER 25 YEARS

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Background / Objectives

In Portugal, cervix cancer has an overall incidence of 13.5 in 100000 women, having its peak incidence in the 4th decade of life. The mortality rate decreased due to an organized screening program, comprising women between 25 and 65 years of age. Organized screening in women under 25 years has not demonstrated any reduction of the incidence or mortality rates. Nonetheless, these women are still screened, occasionally. Based on this fact as well as on the increased occurrence of false-positive screenings in women under 21, it is perfectly adequate that cytological changes or HPV tests are not highly valued. Similarly, between the ages 21 to 25, the follow up of these changes should globally be less invasive, adopting a wait-and-see attitude.

The purpose of this study is to compare the risk factors for cervical cancer between women under 25 and the remaining sample. In this age group, we intend to evaluate colposcopy findings, treatment and follow up.

Methods

Retrospective study of women referred to the Cervical Pathology practice in our Department in 2014, through review of their clinical files (n=356). Statistical analysis made through STATA® v.13.1.

Results

Of the total sample, 5.6% (n=20) of women were younger than 25 years. These presented a statistically significant greater number of partners and an earlier start of their sexual activity when compared to the remaining sample. The most common cytological alteration was low squamous intraepithelial lesion (LSIL) (65%). Upon the first appointment, 30% had a cytology done and 90% a colposcopy, 22.2% of which were normal. Of the 77.8% that had cytological alterations, 78.6% were subjected to a biopsy. Regarding the patients referenced for an LSIL injury, of whatever extent, 100% of the colposcopies found minor anomalies, whereas of those that underwent a biopsy 100% of the histology revealed minor injuries. The most serious injury detected was cervical intraepithelial neoplasia grade 2 (5.5%).

Conclusion
The alterations resulting from the occasional screening in women under 25-years of age continue to account for an increased number of medical appointments. Moreover, these patients present increased risk factors when compared to older patients with cervical pathology. Colposcopy proved to be the favoured exam for the initial approach, with a high number of biopsies. A high concordance between cytology, colposcopy and histological findings was verified, which strongly supports that a less invasive attitude may be adopted. Scheduling appointments with a greater time interval might also be a strategy to avoid over-treatment.

References

Colposcopy Evaluation at the Time of LEEP May Avoid Unnecessary Treatment

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Background / Objectives

The Loop Electrosurgical Excision Procedure (LEEP) is the mainstay technique for the treatment of high-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia (SIL/CIN). The use of colposcopy during LEEP improves the accuracy of treatment and reduces the risks of the procedure. However, its possible benefits in relation to the identification of patients with no lesion at the time of LEEP have not been established. The aim of the study was to assess the accuracy of colposcopy evaluation at the time of the LEEP to identify women with a previous confirmatory diagnosis of SIL/CIN with the absence of dysplasia in the cone specimen.

Methods

We prospectively recruited 162 women undergoing LEEP for histological HSIL/CIN2-3 or LSIL/CIN1 with HSIL cytology showing a fully visible squamocolumnar junction in the colposcopy evaluation at the time of LEEP. At the referral visit cervical cytology, human papillomavirus (HPV) detection and genotype identification, digital colposcopy, size of the colposcopical lesion, and one or more biopsies of the transformation zone were obtained. The uterine cervix was colposcopically evaluated intraoperatively.

Results

Thirty-four women (21.0%) had a normal colposcopy evaluation at the time of the LEEP (study group), while the remaining 128 women showed abnormal findings (control group). Absence of SIL/CIN in the LEEP specimen was confirmed in 28 of the 34 (82.3%) women in the study group and 8 of the 128 (3.1%) women of the control group (p<0.001). A normal colposcopy evaluation at the time of LEEP, lesion size ≤12mm² at the referral colposcopy and HPV genotypes other than 16 or 18 were associated with the absence of CIN in the univariate logistic regression, but only a normal colposcopy evaluation remained significant in the multivariate analysis. A normal colposcopic evaluation at the time of LEEP increased the risk of absence of lesion in the cone specimen 229-fold compared with cases presenting an abnormal colposcopy (95%CI: 33.8-1555.1; p<0.001). The colposcopy evaluation at the time of LEEP had a sensitivity of 87.5% (95%CI: 71.9-95.0) and a specificity of 95.4% (95%CI: 90.3-97.9) to predict the absence of SIL/CIN in the LEEP specimen.
Conclusion

These data show that colposcopy evaluation at the time of LEEP can accurately identify the absence of SIL/CIN before treatment. Thus, the performance of excisional procedures for the treatment of SIL/CIN under direct colposcopy vision should be recommended. Moreover, small lesions and HPV types other than 16 and 18 may point to patients with a higher probability of having a normal colposcopy evaluation at the time of treatment, indicating which women can forgo the treatment.

References


**P21-02**

**IMPROVING CLINICAL PRACTICE: THE EUROPEAN FEDERATION OF COLPOSCOPY QUALITY STANDARDS IN A COLPOSCOPY CLINIC**

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**Background / Objectives**

Quality Assurance (QA) is a way of maintaining a high quality of health care services by constantly measuring the outcome of clinical practice. QA is becoming increasingly important in health care. Nevertheless, there are no specific quality requirements for colposcopy and colposcopy-guided treatments in Switzerland and many other European countries. The European Federation of Colposcopy (EFC) conducted a five-round Delphi consultation to define six quality indicators for colposcopic practice. These indicators were slightly adapted at the EFC general meeting in Paris in January 2017.

**Methods**

We retrospectively evaluated these quality indicators in our colposcopy clinic during the period from January 2015 to December 2016. The six indicators and corresponding targets are (1) documentation of the transformation zone type (100%); (2) percentage of cases having a colposcopic examination prior to treatment for abnormal cervical cytology (100%); (3) percentage of conisations (diagnostic or therapeutic biopsies) with cervical intraepithelial neoplasia (CIN) 2+ (≥85%); (4) percentage of excised lesions with clear margins (≥80%); (5) number of colposcopies personally performed each year with low grade/minor changes (≥50); and (6) high-grade/major lesions (≥50).

**Results**

From January 2015 to December 2016, 148 conisations were performed at our colposcopy clinic. The transformation zone type was documented in nearly every colposcopy (99.3%, 147/148). 99.3% (147/148) had a colposcopic examination prior to treatment for abnormal cervical cytology and 87.3% (130/148) of conisations showed CIN 2+ in diagnostic or therapeutic biopsies. 43.2% (64/148) of excised lesions had clear conisation margins. Each colposcopist at our clinic performed more than 50 colposcopies with low grade/minor changes and high-grade/major lesions per year.

**Conclusion**
Adopting the quality indicators recommended by the EFC offers the possibility to evaluate the performance of colposcopists and provide a benchmark system to secure performance both nationally and internationally. By applying these quality indicators to our retrospective data, we identified our strengths and weaknesses, which will enable us to make future improvements in the care of our patients.

References

A comparison of loop electrosurgical excision procedure using a ring-shaped loop versus a right-angled triangular loop

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Background / Objectives

Objective To compare the resection margin (RM) status and postoperative severe hemorrhage (SH) using different loop electrosurgical excision procedure (LEEP) techniques for cervical intraepithelial neoplasia (CIN) 2/3 treatment.

Methods

Study Design We retrospectively reviewed 278 patients who underwent LEEPs for CIN 2/3 treatment at our institute between 2005–2014. In type A surgery (N=148), a ring-shaped loop was used. If the first pass failed to remove the entire lesion, separate loop excisions for the intracervical portion were performed. In type B surgery (N=130), a right-angled triangular loop in a single pass was used. Surgical outcomes and postoperative SH were compared between the two groups. Logistic regression analysis was performed to identify the independent predictors of RM status.

Results

Results The mean LEEP depth was larger after type A surgery (2.2 vs 2.0 cm, respectively; P=0.04). Type B surgery showed lower rate of 30-day postoperative hemorrhage (13.8% vs 26.4%, P <0.05) and higher rate of negative RM (68.9% vs 82.3%, P<0.05). Multivariate analysis identified the surgery type [P=0.01, OR=0.45 (0.24-0.83)] and a postoperative pathological diagnoses of CIN3 [P=0.01, OR=2.53 (1.22-5.26)] as independent risk factors for positive RM.

Conclusion

Conclusions LEEPs using a right-angled triangular loop could reduce positive RMs.

References

Keywords cervical intraepithelial neoplasia, LEEP, resection margin, postoperative hemorrhage
Post-Coital Bleeding (PCB) as a Predictor for Cervical Pathology: A cross Sectional Study

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Background / Objectives

Post-coital bleeding (PCB) is a disturbing gynecological symptom that may be a concern for both patient and physician, and its reported prevalence varies from 0.7%-9% among menstruating women. PCB may reflect different benign conditions such as infectious morbidities, but can also indicate the presence of cervical cancer. Colposcopy has been suggested as the appropriate investigation tool required for ruling out cervical cancer, or other pre-malignant pathologies; however the literature is not decisive in the management recommendations. The objective of this study was to evaluate the role of PCB in predicting cervical pathology.

Methods

A cross-sectional study using the computerized database of HMO encompassing 2 million insured patients. The research was approved by the local REB committee (number 25/2106). All of PCB cases were identified through a computerized query relating to the years 2012-2016. Further, patients' records in a single center were investigated and the following variables were assessed: age, marital status, ethnical background, gravity, parity, BMI, smoking, socio-economic status, past history of cervical pathology, PAP smear result and colposcopy evaluation. Colposcopy reports were reviewed for findings, required biopsy and biopsy results. For the purpose of this study we included non-pregnant patients between 18-50 years.

Results

Incidence/100,000 patients during the study period ranged 326.1-565.9 cases/year. Among investigated records, mean age was 32±7.9 years, mean BMI: 24.0±4.3, 53% were married, 49.1% gave birth, 17% were smoking and 18.7% presented with a background medical diagnosis. 8% of PCB cases had an abnormal PAP smear in the preceding year. All sample cases went through a colposcopy by a single practitioner; 201 (48.9%) requiring a biopsy. Biopsy results were as following: 44 (21.9%) normal tissue, 25 (12.4%) cervical polyp, 68 (33.8%) cervicitis, 61 (30.3%) HPV-related/CIN 1/condylomas, 2 (1.0%) CIN-2/3 and 1 case (0.5%) of
carcinoma. The positive predictive value for HPV-related pathology was 15%, and for high-grade lesions (CIN-2/3 and carcinoma): 0.7%. In a multivariate logistic regression analysis, parity and the presence of a pathological PAP smear (P value 0.02, OR 0.39 and P value 0.01, OR 3.3 respectively) were significantly related to HPV-related cervical pathology.

Conclusion

PCB is a common gynecological complaint with relatively high prevalence of HPV-related pathologies. Although high grade lesions are rare, we recommend considering colposcopic evaluation in those women.

References
Background / Objectives

The aim of the study was to analyze the regression, persistence and progression of cervical intraepithelial neoplasia first diagnosed during pregnancy, in order to assess the suitable management of such lesions for the pre- and postpartal period.

Methods

In the course of this study the cases of 138 pregnant women who presented with pathological cervical findings at the Dysplasia Clinic of the University Medical Center Hamburg Eppendorf between the years of 2011 and 2017 were retrospectively analyzed. Differential colposcopy, a cytology, a biopsy and as appropriate a HPV test were performed on all patients. In the case of CIN diagnosis regular follow-ups were carried out. The initial histopathological findings were compared to those of the postpartal period.

Results

A total of 138 pregnant women of the median age of 31 years (range 19-41) with colposcopic evidence of cervical dysplasia (n=15) or suspicious cytology (n=53 with PAP IIID, n= 70 with PAP IVa/b) were included. On average the patients first presented in the 17th (range 5-31) week of pregnancy and were followed-up every 8 weeks. No progression to carcinoma was diagnosed in any of our patients and no woman had to be subjected to a conisation during the course of pregnancy. 60 patients with initial CIN diagnosis during pregnancy were presented for a scheduled postpartal exam, where 16.7% (n=10) showed a partial regression of CIN, while 40% (n=24) showed a complete regression of CIN. 33.3% (n=20) were diagnosed with persistent findings of CIN. In 10% (n=6) of cases progression to severer CIN not however to a carcinoma had occurred. All in total 34 patients were operated on postpartally by conisation and endocervical curettage.

Conclusion

CIN lesions during pregnancy have a prepartal slight progression and postpartal a higher tendency for regression. After the exclusion of an invasive procedure, the definitive treatment can be postponed with little risk to the postpartal period. The necessity of a check-up every 8 weeks after the detection of a high-grade lesion (CIN 2-3) during pregnancy can not be deduced/inferred from these findings.
ASSOCIATION BETWEEN VAGINAL MICROBIOME, HIGH RISK HPV PROFILE, HPV E6/E7 RNA EXPRESSION AND SEVERITY OF CERVICAL PRECANCEROUS LESIONS

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Background / Objectives

Role of persistant HPV infection in development of precancerous lesions is essential and multiple HPV infection correlation with E6/E7 RNA expression is shown in some studies (Anderson 2012). There are no studies in Latvia on HR HPV profile and E6/E7 RNA expression in patients with cervical intraepithelial neoplasia.

Methods

49 women aged 18-65 with abnormal cytology referred for colposcopy during their first visit to Reference Colposcopy Centre in Riga East Clinical University Hospital in July 2016-February 2017 were included in the study. Results of vaginal pH, native microscopy of frontal vaginal fornix, material from cervix for presence of high risk (HR) HPV DNA types 16/18, 31, 33, 45, 58, HR- HPV E6/E7 common RNA and histology after punch biopsy taken under colposcopy control were analyzed for each patient. Vaginal pH was measured using Machery Nagel pH strips. Microscopic examination of wet mounts was interpreted according to Donder’s modification of Schröder’s classification. HPV types and HPV RNA were identified by real time PCR test.

Results

14 patients with low grade squamous intraepithelial lesions (LSIL), 32 with high grade SIL (HSIL), 2 patient with atypical squamous cells of undetermined significance (ASCUS) and 1 patient with atypical glandular cells of undetermined significance (AGUS) were included in the study. HR- HPV DNA was detected in 26 cases (HPV positive group), 23/26 was multiple HPV infection. The most common HPV type was HPV 58, which was isolated from 23 women, HPV16/18 was found in 16 patients, HPV 31 in 13 cases and 33 in 11 patients, HPV 45 in 11 patients. Elevated pH >4.4 was detected in 12/26 patients from HPV positive group and in 5/23 cases from HPV negative group (p=0.02). Lactobacillary grade III prevalence in both groups did not differentiate significantly in our study, 7 cases in HPV positive group and 8 cases in HPV negative group. HPV E6/E7 RNA expression was found in
26/26 cases of HR-HPV DNA positive group and in 13 cases of HPV negative group (p<0.01). Multiple HPV types were found in 19/34 patients with CIN 2+ histology. CIN2+ in histology reports was more likely correlate with HPV E6/E7 RNA expression in both groups: 21/26 patients in HPV positive group and 13/13 cases from HPV DNA negative group (p=0.05).

Conclusion

Our findings suggest a possible association between multiple HPV DNA, E6/E7 RNA expression and high grade cervical precancerous lesions, but we analyzed only 6 HR-HPV types and E6/E7 RNA prevalence in HPV negative group may be associated with other HR–HPV types. More detailed study will be required in future.
THE DISCOVERY OF A ANTITUMOR ACTIVITY OF THE ALKALOID ERYTHRALIN: INDUCTION OF APOPTOSIS IN SIHA CELLS BY ARRESTING THE CELL CYCLE AT THE G2-M PHASE

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Background / Objectives

Cervical cancer is the fourth leading cause of cancer death in women worldwide and persistent infection with a high risk human papillomavirus (HR-HPV) is the main etiological factor. Several studies have sought to identify compounds with selective activity for tumor cells that have an apoptotic mechanism of action, therefore it is important investigate bioactive new chemical entities mainly from biodiversity. *Erythrina velutina* (EV) is a plant native from Brazil popularly known as mulungu. Seeds and barks are used in folk medicine as sedative, anticonvulsant and in sleep disorders. Among the metabolites found in the genus it is highlighted the occurrence of erythrinic alkaloids in several species. In this study, the alkaloid Erythralin was evaluated for anti-tumor activities against human cervical carcinoma cell line (SiHa).

Methods

Cell viability was quantified by the MTT assay and absorbance (570 nm) by an ELISA reader, in each experiment. The apoptotic cells were evaluated using Propidium and Annexin Iodide staining and analyzed by flow cytometry. Nuclear morphological changes were evaluated by fluorescence with DAPI staining and flow cytometry was used to cycle assay.

Results

The alkaloid Erythralin significantly inhibited (p <0.05) the growth of SiHa cells after 24 and 48 hours. The cell viability assay showed that the inhibitory effects of Erythralin were also consistent with the morphological changes observed under light microscopy in a dose-dependent manner. There was also an increase in apoptotic cells in a dose-dependent manner through cytometric analysis. This is the first study that
demonstrated cytotoxic and pro apoptotic effects of Erythralin on SiHa cells. The results also suggest a tendency to stop the cell cycle in the G2-M phase.

**Conclusion**

Preliminary studies on mechanism reveal that Erythraline induces apoptosis in SiHa cells by arresting the cell cycle at the G2-M phase. However, further evaluations are necessary for the evaluation of its antitumor properties and mechanisms of action. Our results suggest that compound *E. velutina* might be a potential candidate for developing novel anti-cancer drugs in the coming future.
P22-06
CITOTOXIC AND PRO APOPTOTIC ACTIVITIES OF CROTON BLANCHETIANUS IN HUMAN CERVICAL CANCER HELA AND SIHA CELLS

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Background / Objectives

Cervical cancer (CC) is the third most common cancers in women worldwide and the fourth major cause of cancer death in the woman in developing countries, remaining a critical public health problem. High-risk human papilloma viruses (HPVs) such as HPV 16, 18, 31 and 33 have been attributed to be the major risk factors for cervical cancer. Platinum based chemotherapy in combination with radiotherapy or surgery is now mainly used to treat CC, but the efficacy is limited especially in advanced-stage disease. Furthermore, these treatments could easily lead to adverse reactions and drug resistance. Therefore, the discovery of new highly selective and efficacy drugs has been the main focus of the research. Thus, the study aimed to investigate, in vitro, the cytotoxic and pro apoptotic effects of leaves and roots fractions from Croton blanchetianus (CB) against human cervical cancer HeLa and SiHa cells.

Methods

Samples were obtained from a crude ethanolic extract after acid-base extraction with chloroform at pH 2 (CBaF from leaves; CBaR from roots) and at pH 9 (CBbF from leaves; CBbR from roots). Phytochemical screening was evaluated by thin layer chromatography using Sulfuric Vanilin, Dragendorff and Natural A Reagent as stain. Cytotoxic activity and apoptosis rates were determined with MTT and Annexin V/PI assays, respectively. Nuclear morphological changes were evaluated by fluorescence with DAPI staining and flow cytometry was used to cycle assay.

Results

According to results, all fractions exhibited terpenoids, alkaloids and flavonoids, except CBbF that showed no flavonoids. All fractions decreased significantly cell viability of HeLa and SiHa in a concentration- and time-dependent manner (p<0.05),
as well as, they induced cellular and nuclear morphological changes, apoptosis and cell cycle arrest (p<0.05).

**Conclusion**

This is the first study that demonstrated cytotoxic and pro apoptotic effects of *Croton blanchetianus* on HeLa and SiHa cells. Therefore, *Croton blanchetianus* appears to be a valuable natural source for the development of agents for the treatment of cervical cancer. However, the present study points to the need for further phytochemical research to isolate the biologically active products of these fractions responsible for the observed activities and to elucidate their action mechanisms.
Background / Objectives

Vulvar intraepithelial neoplasia (VIN) is a premalignant pathology which leads to vulvar carcinoma, the fourth most common gynaecological cancer. Although invasive vulvar cancer rate has remained the same in the last two decades, VIN incidence has doubled. To date, no screening programme exists for early diagnosis of vulvar HSIL, the diagnosis being based on clinical findings and confirmed by biopsy. The aim of this study was to demonstrate if HPV is the carcinogenetic factor incriminated in vulvar cancer.

Methods

During 2011-2016, in the Obstetrics & Gynaecology Clinical Hospital "Panait Sirbu" Bucharest, 20 VIN patients were diagnosed. There were also 12 vulvar cancer which after the confirmation of squamous carcinoma diagnosis, received standard surgical treatment. 9 patients were HPV-PCR tested from biopsy tissue. Surgical treatment was rendered in oncology hospitals with good functional results and no local relapses. HPV infection was present in all 9 biopsy tests, high risk strains 16,18,31,35 and 51 being most commonly found. 6 patients also had positive cervical HPV testing.

Conclusion

Although VIN is not very frequent, because of the appearance on exophytic and endophytic lesions and even vulvar distrophy, the carcinogenetic factor – HPV, should be systematically tested. Early surgical treatment of confirmed vulvar cancer has the best prognostic.
ETIOLOGICAL ROLE OF HUMAN PAPILLOMAVIRUS INFECTION IN THE DEVELOPMENT OF PENILE CANCER

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Background / Objectives

We investigated an etiological role of HPV infection in the development of penile carcinoma.

Methods

Paraffin-embedded tumor samples were collected from 17 patients who had received an operation for penile carcinoma. After DNA extraction from each sample, HPV-DNA test and genotyping were performed using a HPV GenoArray kit (Hybri MaxTM). In addition, localization of HPV was observed by in situ hybridization (ISH) for high-risk HPV-DNA. Furthermore, P16-INK4a and HPV-L1 capsid protein expression were evaluated by immunohistochemistry (IHC).

Results

HPV-DNA was detected in 7 (41%) cases; HPV16 was identified in 5 samples, HPV33 and HPV68 was identified in one case, respectively. ISH analysis demonstrated that high-risk HPV-DNA was localized with punctate staining patterns in the nuclei of tumor cells of all HPV-positive samples. P16-INK4a was moderately to strongly expressed in nuclei and cytoplasm of tumor cells in many of HPV-positive samples, whereas showed the relatively weak or no expressions in HPV-negative ones. On the other hand, HPV-L1 protein expression, which suggested reproductive HPV infection, was not observed in any carcinoma.

Conclusion

The current results suggest that high-risk HPV, especially HPV16 is likely to be a causative agent among an approximate 40% of the Japanese patients with penile
carcinoma, although further studies including a large number of samples are required to reach a more definite conclusion.

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Cooper K, Herrington CS, Stickland JE, Evans MF, McGee JO. Episomal and

FACTORS ASSOCIATED WITH ABNORMAL ANAL CYTOLOGY OR HR-HPV ANAL INFECTION AMONG HIV POSITIVE MSM

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Background / Objectives

Anal cancer is caused by human papillomavirus (HPV), which can cause changes to the skin around and mucous epithelium inside the anus. Although in the general population anal cancer is a relatively rare neoplasia with incidence between 1 to 2 cases per 100,000 people, estimates of the anal cancer rates among HIV positive men who have sex with men (MSM) range from 70 to 137 cases per 100,000. Anal cytology and HPV detection are important tools for the anal cancer screening. Aim of this study was to compare patient’s characteristics with the anal cytology results.

Methods

In this study HIV positive MSM attending Dermatovenereology Department of Nemocnice Na Bulovce who signed an informed were included. Anorectal cytology and HPV specimens sample were taken from the entire length of the anal canal mucosa using a moistened Dacron swab. After sampling, cells from the swab were washed into a vial with liquid-based cytological medium. In the cytological laboratory, samples were processed to evenly disperse as a thin monolayer of cells while removing background obscuring materials. The presence of anal HPV-DNA was detected by PCR with broad spectrum primers followed by hybridization.

Results

The average age of the 80 HIV positive MSM who agreed to participate in the study was 33.8 years (range 23-57). In our study 65 (81.3%) of the patients were on highly active antiretroviral therapy (HAART) for more than 3 months prior to the specimen collection. Average CD4 cell count of the patients at the time of the study was 671 cells/mm3 (range 361-1180). HPV-DNA was detected among 71 (88.8%) of the patients. HR-HPV infection was present among 47 (58.8%) patients. Low-grade squamous intraepithelial lesion (LSIL) was detected in 34 (42.5%) patients, normal cytology (NILM) had 23 (28.8%) patients and none of the patients had high-grade squamous intraepithelial lesion (HSIL). HR-HPV anal infection was significantly more common among patients under 34 years of age (73.3% vs. 40.0%; p < 0.01). Presence of abnormal cytology was not associated with patient’s age, HAART or CD4 cell count.

Conclusion
Our preliminary results suggest that anal HR-HPV infection is more common among younger HIV positive MSM. We did not observed association between the presence of HR-HPV infection and HAART or CD4+ cell count over 350. Similarly abnormal cytology among HIV positive MSM in our study was not associated with these factors. Because the results are based on the preliminary data obtained from a small number of patients and none of the patients had CD4 cell count under 350 cells/mm3 they should be interpreted with caution.
P25-02
HPV genotyping and E6/E7 HPV mRNA expression analyses in anal cytology samples for prevention of HPV-related anal cancer.

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Background / Objectives

Anal cancer incidence is high in populations at high risk, as HIV-infected men who have sex with men, receptive anal intercourse, and high risk sexual behaviour, warranting consideration of early detection approaches. HPV has been shown to be a major cause in the development of anal cancer: persistent infection with high-risk types of HPV causes more than 80% of anal cancers. In the precursor to anal cancer, anal intraepithelial neoplasia (AIN), the prevalence of HPV infection is high. There is no widely accepted procedure guidelines for men with possible exposure to HPV that can lead to dysplasia. The aim of this study is to determine the prevalence of HPV DNA and RNA, to lay the bases for a possible screening strategy for the prevention of anal cancer in high-risk populations.

Methods

We evaluated anal pap test and HPV infection, with both HPV genotyping and mRNA HPV tests, in 129 anal cytology samples collected at the center of the National Surveillance Network of Sexually Infections of Florence in the period 2015-2016, and sent to ISPO for the analyses. The specimens were collected in ThinPrep vials (Hologic). HPV typing was performed by reverse line hybridisation (Ampliquality HPV express AB analitica). Genotype analysis for HPV DNA was available for 52 samples. RNA analysis for E6/E7 HPV mRNA expression was available for 26 samples. The test was performed with Aptima HPV assay (Hologic) with Panther system, that qualitative detects 14 hr- HPV type. 27 samples were also tested for HR-HPV DNA by Cobas 4800 HPV test (Roche).

Results

The prevalence of HPV infection is 78.8% if considering both high risk and low risk HPV types, and 57.7% if considering only hr types. The most prevalence type is HPV16 pos (26.8%), followed by HPV 45 (22%) and HPV 18 (19.5%). Multiple
infections are frequent: more than 3 types are co-infecting in 25 samples. mRNA HPV was positive in 65.4%. All mRNA-negative samples have negative pap test or non valuable. Of the 45 samples with cytology ≥ ASC-us, 24 were tested for HPV Typing: 79.2% were positive for hr-types; 47.4% of these were HPV16-positive. Cobas HPV test results are concordant (100%) with typing results.

Conclusion

In order to establish a pilot screening programme for anal cancer, it is valuable to determine the prevalence of HPV types and the expression of viral oncogene E6/E7. Anal cytology has been used to predict those at risk of AIN, but the limited sensitivity restricts its usefulness as a potential screening technique. Completing HPV DNA e RNA analyses on all samples, and data from follow up on this group of patients will determine if HPV tests (DNA or RNA) can be applied in a screening contest for anal cancer prevention.
P25-03
ANAL INTRAEPITHELIAL NEOPLASIA (AIN) AND
ANAL SQUAMOUS CELL CARCINOMA (SCC) IN A
LARGE URBAN COHORT OF HIV-POSITIVE
INDIVIDUALS LIVING IN THE UNITED KINGDOM: A
RETROSPECTIVE DATA ANALYSIS

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Background / Objectives

HIV is associated with a 30-fold increased lifetime risk of anal SCC and a 4-fold increase in 5-year mortality. Men who have sex with men (MSM) with HIV are at increased risk of human papillomavirus (HPV) associated cancers including anal SCC. Diagnosis of AIN presents an opportunity to initiate monitoring before SCC development. The characteristics of patients with AIN are poorly understood, as are the factors associated with progression from AIN to SCC. This project aimed to describe the cases of AIN and anal SCC in a large urban cohort of people living with HIV (n=2400) in the UK.

Methods

We identified all cases of AIN and anal SCC in patients attending a single HIV outpatient centre in the UK. We reviewed case notes and histopathology.

Results

23 AIN cases and 28 SCC cases diagnosed 2001-2016: all white MSM. Where documented, 56% were current smokers and 36% ex-smokers. Median age 48 years (range 27-73), nadir CD4 284 cells/mm3 (4-1312), median months since diagnosis 171 (24-361), 100% on antiretroviral therapy (ART). Of the AIN group 16/23 (70%) had previous anorectal sexually transmitted infections (STIs), 15/23 (65%) HPV, 5/23 (22%) gonorrhoea, 4/23 (17%) herpes simplex virus (HSV) and 2/23 (9%) chlamydia. Of the SCC group 24/28 (86%) had documented STI history, 21/24 (88%) with anorectal STIs: 16/21 (76%) HPV, 5/21 (24%) gonorrhoea, 4/21 (19%) chlamydia and 4/21 (19%) HSV. Most AIN patients (83%) presented with an anal lump and the majority (83%) were AIN III. Patients who progressed from AIN to SCC did over 1-9 years, had comparable age (median 52, range 39-72) and nadir CD4 385 cells/mm3 (4-1312) to the broader cohort but were diagnosed with HIV further in the past (210 months,159-226). SCC presenting symptoms were anal lump (75%), pain (21%), and rectal bleeding (17%). 25/28 (89%) had local disease, 3/28 (11%) local nodes with no metastatic disease, 4/28 (14%) had previous AIN. Of those treated at our centre 8/13 (62%) had chemoradiotherapy, 2/13 (15%) had radiotherapy alone, and 3/12 (23%) had surgery. 2/13 (15%) patients needed surgery after unsuccessful chemoradiotherapy. 2/14 (14%) diagnosed with AIN after
successful SCC treatment. 5/28 (18%) patients have died, with 2 deaths attributable to SCC.

Conclusion

AIN and SCC are emerging issues for MSM living with HIV on effective ART. We found that a large proportion of patients had anorectal HPV diagnosed before anal SCC but only a minority had previously diagnosed AIN. Further research is needed to clarify which patients are most at risk of developing SCC and to establish the impact of anal cancer screening on the reduction of anal SCC in this population is urgently needed.
Background / Objectives

The incidence of anal cancer is increasing worldwide, but limited information is available about the prognosis of these tumors. Human papillomavirus (HPV) DNA have been investigated as a prognostic factor in anal cancer. The objective of this study was to retrospectively investigante HPV DNA and clinical data in a series of consecutive cases of invasive anal carcinomas treated in a single institutional service in Brazil. HPV DNA prevalence and genotype distribution were investigated in association with clinicopathological characteristics.

Methods

A group of 81 patients with invasive anal carcinomas was retrospective analyzed for a period of 10 years. Formalin fixed paraffin- embedded samples were tested for HPV detection and genotype distribution by using a SPF-10 Inno Lipa assay. Prevalence ratios were estimated by logistic regression and survival was analyzed by Kaplan Meier and log-rank.

Results

The prevalence of HPV DNA for the whole group was 69%, and it was significantly higher in squamous cell carcinomas (SCC) (88.1%) (OR 9.51 IC 95% 2.96-30.50) and in female patients (78.4%) (OR 3.18 IC 95% 1.19-8.48). Multiple infection was detected in 14.3% of cases and HPV 16, 18 and 33 were the most prevalent genotypes. Overall survival for the group was 44.3%. Survival was significantly higher for men (p = 0.008) and for SCC patients (p = 0.01), and was reduced for patients with distant metastasis (p = 0.01). HPV positive tumors presented with a higher survival, although the difference was not significant (p > 0.05).

Conclusion

The high prevalence of HPV DNA in anal carcinomas was confirmed in this study, however, the presence of HPV DNA did not significantly affect the prognosis of the patients. Prognosis was affected by gender, histological type and distant metastasis.

References
P26-01
HPV DETECTION IN ORAL CAVITY OF ASYMPTOMATIC PEOPLE FROM NORTH ARGENTINA

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Background / Objectives

Infection with HPV is clearly associated with epithelial alteration of genital tract and its oncogenic role is undoubtedly linked to cervical cancer (CC). By contrast, the relevance of the presence of HPV in other sites, like oral cavity, is still poorly studied in major regions of the world with historically high incidence of CC. The aim of our study is to better understand the transmission dynamics of HPV in oral cavity of unvaccinated people from Chaco, a north region of Argentina with a high prevalence of cervical HPV infection and CC. This ongoing research work is feasible thanks to the funds granted by the National Cancer Institute of Argentina.

Methods

Our cross-sectional and observational study shows the results of the first 266 samples of a total of 500 that will be collected by december 2017. Oral rinse/gargle samples were obtained from asymptomatic and sexually active volunteers (women and men). People recruited were from different areas of Resistencia city (Chaco, Argentina), guaranteeing an adequate population heterogeneity and age distribution. A standardized questionnaire was used to interview the participants regarding their clinical history, sexual behavior, cultural habits and socio-economic and living conditions (data not shown). DNA of each sample was extracted and purified by a commercial kit (High Pure PCR Template Preparation Kit, Roche) and the presence of HPV was analyzed by the well known PGMY09/11 general PCR. Positives ones were further studied for HPV genotyping with a commercial kit (HPV Direct-Flow Chip) produced by Master Diagnostica (Vitro Group, Spain).

Results

To date, we have analyzed 266 oral rinses, 118 from men (44,4%; mean age 38 years) and 148 from women (55,6%; mean age 40 years). Six samples (2.3%) were positive for HPV (only in men). There were three cases with a unique HPV detected (HPV-16, -61 and -44/55 respectively); two with multiple genotypes (among them HPV-18, -6, -62/81, -39, -61) and one sample with a non-typifiable HPV type. Regarding the variables possibly associated with HPV infection, it is necessary to finish the samples collection and analysis projected for this work, to conclude properly about them.

Conclusion
HPV infection in the oral mucosa is currently a topic of great interest in many regions of the world. Our study is a contribution to estimate a basal line of oral HPV infection in asymptomatic people. Data collected will be important to analyze, in future studies, the impact of HPV infection in recognized risk-groups (People living with VIH, immunosuppressed, oncology patients, etc) and to evaluate the global impact of the current HPV vaccination strategies in Argentina.

References

PREVALENCE OF HPV-16 AND 18 IN PATIENTS WITH ORAL LEUKOPLAKIA: A PRELIMINARY MOLECULAR AND IMUNOHISTOCHEMICAL STUDY FROM A BRAZILIAN COHORT.


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Background / Objectives

Studies have shown that HPV might play a role in the pathogenesis of a portion of oral leukoplakia (OL) cases, nevertheless, highly variable HPV detection rates in this disorder suggests the need for further researches aiming to elucidate which factors might be involved in the development of OL, better explaining the participation of HPV in this process. Therefore, the aim of this study is to evaluate the presence of HPV-16 and HPV-18 DNA, which is the oncogenic genotype of greater relevance, in different biological samples from patients with oral leukoplakia, and the correlation of these factors with sociodemographic and clinicopathologic characteristics and prognosis of OL.

Methods

Forty patients with OL will compose the study group, and a control group of 40 healthy patients requiring pre-prosthetic oral surgery will be matched to the patients of the study group by sex and age. Tissue, saliva, and blood plasma samples will be obtained from patients of both groups. HPV-16 and HPV-18 DNA detection will be performed in the tissue, saliva, and plasma blood samples from both groups by Real Time PCR (RT-PCR) technique. Furthermore, immunohistochemistry analysis will be performed for p16INK4A in paraffinized tissue samples for evaluating the presence of HPV and the activity of the virus.

Results

Until this moment, a pilot study was performed analyzing the HPV-16 detection among 5 OL patients. Descriptive analysis of clinicopathologic features of these patients demonstrated that most of patients were male (60%), and age ranged from 45 to 66 years old (average = 55.2). Two (20%) were old-aged, 40% were middle-aged, and 40% were young. All patients were current smokers (100%), while none was never or ex-smoker. Most of patients with OL were current drinkers (80%), and 20% were ex-drinkers, while none was never-drinker. 60% of the 5 OL lesions analyzed until this moment displayed some degree of epithelial dysplasia. HPV-16 DNA was not present in none (0%) of fresh tissue and saliva samples from the 5 patients included in the study until now.
Conclusion

No relevant conclusion is possible at this moment. However, further analyses are necessary for better understanding this preliminary study.

References


DNA DAMAGE RESPONSE AS TUMOR SELECTIVE RADIosenSITIZATION STRATEGY FOR HPV POSITIVE AND NEGATIVE HEAD AND NECK CANCERS

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Background / Objectives

Treatment of head and neck squamous cell carcinoma (HNSCC) is characterized by high local recurrences mainly due to the DNA repair capacity of cancer cells. Selectively inhibiting these DNA repair mechanisms in combination with radiotherapy (RT) could increase locoregional tumor control. Moreover, recently we found that human papillomavirus positive (HPV+) HNSCC cells have differences in their DNA repair efficiency compared to HPV- cells, suggesting differences in mechanisms of DNA repair and showing the need for different treatment approaches for HPV+ and HPV- HNSCC.

Methods

We performed a CRISPR/Cas9-based loss of function screen targeting 36 drugable genes in DNA damage response (DDR) to discover the most efficient therapeutic targets that are synthetically lethal in combination with RT for either HPV+, or HPV- tumors. We validated these results with commercially available drugs (NU7441 (DNA-PK inhibition); ABT-888 (PARP1/2 inhibition); AZD7762 (CHK1/2 inhibition)) by survival and clonogenic assays. We investigated the repair kinetics by gH2AX and RAD51 foci formation and the effect on cell cycle by flow cytometry. The therapeutic efficacy in cell line based and PDX mice models was assessed by tumor growth delay curves.

Results

Our results revealed 14 hits for HPV+ and 18 hits for HPV- HNSCC, showing the presence of differences in DDR between HPV+ and HPV- HNSCC. Inhibition of PARP radiosensitized HPV+ HNSCC cells. This effect was due to p16-mediated inhibition of homologous recombination repair. This p16-dependent effect also translated in a slower tumor regrowth in vivo models. CHEK genes were important for survival of HPV- HNSCC. The majority of the overlapping genes were involved in the non-homologous end-joining pathway. Validation of these synthetic lethal hits with DNA-PK inhibitor (NU7441) radiosensitized HPV+ and HPV- HNSCC in vitro and in vivo.
Conclusion

Our results lead to robust assessment of novel targeted radiosensitizers for HPV+ and HPV- HNSCC.
PREVALENCE AND IMPACT ON SURVIVAL OF HPV AND P16 IN OROPHARYNGEAL CANCER OTHER THAN TONSIL OR BASE OF TONGUE CANCER

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Background / Objectives

Today, most oropharyngeal squamous cell carcinoma (OSCC) is human papillomavirus (HPV) positive and HPV alone or in combination with p16 is reported to be a favorable prognostic factor for OSCC. Patients with tumors at other OSCC sites (OOSCC) are often included in the same treatment and study protocols as patients with tonsillar- and base of tongue SCC, even though the prevalence and clinical significance of HPV infection and the correlation to p16 in OOSCC still is unclear. Since tonsillar and base of tongue SSC cover roughly 90% of all OSCC, there is an obvious risk that there may be a misinterpretation of the results for OOSCC. We have in a previous minor study of 69 patients with OOSCC shown that only a minority (16%) to be HPV positive and 25% to be p16 positive. In addition, there was no complete correlation between HPV status and p16. Furthermore, no impact was seen on clinical outcome for the HPV-positive patients. We are therefore investigating the prevalence and correlation of HPV and p16 in OOSCC and their impact on survival a larger cohort from the Karolinska University Hospital, including patients from 2009-2014. This is of special interest since the International Union Against Cancer (UICC) in their eight edition changed the TNM-classification for p16-positive tumors for the whole OSCC-group.

Methods

All OSCC patients (C10.0–C10.9 and C50.1–C50.8) diagnosed between 2000 and 2014, in the County of Stockholm, Sweden, were included in the study. HPV-DNA was detected by PCR and p16 by immunohistochemistry. The study was conducted according to ethical permissions 2005/431-31/4 and 2005/1330-32 and 2009/1278-31/4 from the Ethical Committee at Karolinska Institutet, Stockholm, Sweden.

Results

Preliminary results: 108 patients have been included so far (2000-2012) so far and of those 22 (20%) had HPV positive tumors. 68 tumors have been tested so far and 11
(16%) were p16 positive. Of the 22 HPV positive tumours, 21 were also tested for p16 and only 8/21 (36%) were positive for both p16 and HPV.

**Conclusion**

Preliminary results show that the of HPV and/or p16 is much lower in OOSCC compared to earlier reports including all OSCC, or tonsillar and base of tongue cancer alone, the correlation between HPV-status and/or p16 in the tumors was not as strong as shown in previous studies including all OSCC or tonsillar SCC. We suggest that HPV/P16-positive OOSCC should not be treated in a similar way to HPV/p16 positive tonsillar and base of tongue cancer until larger studies have clarified the discordance between HPV and p16 overexpression and their clinical impact.
COMPARISON OF ANYPLEX II HPV 28 AND SPF10/DEIA/LiPA25 IN FFPE OROPHARYNGEAL CANCER SAMPLES

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Background / Objectives

Human Papillomavirus (HPV) infection has emerged as a major etiological factor with a prognostic significance in oropharyngeal squamous cell carcinomas (OSCC). The identification of HPV-related tumors in the clinical setting is still mainly based on the p16INK4a overexpression. However, a recently published meta-analysis (Prigge ES, IJC 2017) shows that the combination between p16 overexpression and HPV DNA PCR testing increases the specificity to distinguish HPV-related OSCC. Several techniques focused on HPV DNA detection and genotyping, which have been developed and used for prognosis purposes, could also be used in the near future for treatment decision-making. Here, we compare the results obtained analyzing formalin-fixed paraffin-embedded (FFPE) OSCC tissue blocks using the Anyplex II HPV 28 test and two different extraction methods with the results previously obtained using the SPF10/DEIA/LiPA25 combination for HPV DNA detection and genotyping.

Methods

FFPE samples were selected from an international collection including 1090 OSCCs subjected to histopathological evaluation, HPV-DNA, HPV E6*I mRNA and p16INK4a detection (Castellsagué/Alemany, JNCI, 2016). A subset of 95 FFPE samples were randomly selected from strata specified (40 HPV DNA negative; 40 HPV DNA positive + mRNA or p16 positive; 12 HPV DNA negative+p16 positive; 3 HPV DNA positive + mRNA/p16 negative). DNA extraction for HPV DNA detection (SPF10/DEIA/LiPA25) was performed using a Proteinase K digestion method as previously described. In the present study, samples have been retested using Anyplex II HPV 28 test and two
different DNA extraction methods. Proteinase K digestion method and DNA extraction using the MagCore automatic station were compared. We calculate the agreement between the SPF10/DEIA/LiPA25 and Anyplex II HPV 28 test.

Results

Preliminary data obtained after the analysis of 32 samples show 100% agreement between SPF10/DEIA/LiPA25 and Anyplex II HPV 28 tests. Anyplex II HPV 28 test was positive for 58% and negative for 42% of samples. All HPV positive samples showed the HPV16 type. A complete agreement in HPV detection and genotyping was obtained using Proteinase K digestion method and the MagCore protocol.

Conclusion

Preliminary data have shown a high agreement between SPF10/DEIA/LiPA25 and Anyplex II HPV 28 tests for HPV detection and typing in FFPE oropharyngeal samples. This is an on-going study and complete results will be presented in EUROGIN 2017 congress.

References


Background / Objectives

There is growing evidence that human papillomavirus (HPV), in particular HPV16, may be involved in development of some head and neck (HN) squamous cell carcinomas (SCC). However, the prevalence of HPV and its prognostic potential is still the subject of worldwide discussion. Therefore, the aim of our study was to assess the frequency of HPV, its type and prognostic role in patients with HNSCC from south-central Poland.

Methods

The study was carried out in the group of 113 patients with SCC of oral cavity, pharynx or larynx. Experiments were performed using DNA isolated from formalin-fixed paraffin-embedded tumour samples. HPV infection was assessed using nested PCR with PGMY/GP+ primers (according to our best knowledge for the first time in Poland) based on 3-4 experiments for each tissue. Virus type was analyzed by real-time PCR.

Results

DNA was obtained from the material of 109 patients. Based on nested PCR results we qualified 60 (55.0%) of 109 tumours as HPV positive and the infection was confirmed by real-time PCR in 39 (35.8%) of them. The proportion of HPV infection was the highest in oropharyngeal tumours (48.5% of positive cases; 33/68), whereas in oral and hypopharyngeal ones were 20.8% (5/24) and 14.3% (1/7) respectively. None of laryngeal cancer (0/10) had viral infection. HPV16 was the predominant type (82.1%). We also identified 3 cases of HPV35 and 4 of dual infection (HPV35 together with HPV16 or HPV18). The experiments are ongoing and complete data concerning correlation between HPV presence and patients’ survival will be presented during conference.

Conclusion
Some head and neck cancers (mostly within oropharynx) in south-central Poland seem to be HPV-driven, mainly as a result of HPV16 infection, less often because of HPV35 or HPV18.

The study was partially financed by the National Science Centre, Poland project No. 2016/21/N/NZ5/00227.
HISTOLOGICAL SUBTYPE OF SQUAMOUS CELL CARCINOMA OF HEAD AND NECK AND THE PRESENCE OF HPV ASSESSED BY THE IMMUNOXESSION OF P16

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Background / Objectives

Head and Neck Squamous Cell Carcinoma (HNSCC) is a malignant disease entity with a high prevalence in the world population (1). Among its major risk factors, there is a persistent infection with Human Papilloma Virus (HPV), which has been related to a better prognosis in patients. HPV infection results in an immunoreactivity of p16 protein that has been used as a marker of the oncogenic lineage by this etiologic agent (3,4). Objective: To analyze epidemiological aspects of patients affected by HNSCC (age, sex and location of the lesion), and relate them to the prevalence of HPV infection. To evaluate the presence of virus stigmas the samples (koilocytes). To correlate histological types and differentiation of HNSCC positive for p16

Methods

A cross-sectional and retrospective study in the electronic archives of the Hospital Universitário Evangélico de Curitiba, in cases with diagnosis of Oropharyngeal SCC, which occurred between January 2005 and December 2015. Slides stained by the HE technique were reviewed and classified histological type of the lesion and verified of histological stigmas characteristic of HPV (koilocytes). Squamous cell carcinomas (SCC) were classified in keratinizing, no keratinizing and mixed. The paraffin blocks were screened to select the sample areas for the preparation of tissue microarrays (TMAs) in which was performed immunohistochemical study of the p16 protein by avidin-biotin technique. All results and information obtained were tabulated according to data protocol, and then expressed through graphs and tables and statistical analysis was performed by parametric and non-parametric methods, with significance of p <0.05 . The project, was approval by the Research Ethics Committee of the Evangelical Society of Paraná

Results

Of the 51 cases evaluated, 42 were males and 9 females, mean age of 61 years. There was a higher percentage of tumors affecting the larynx (43%), with higher prevalence of keratinized cancers on non keratinized. Koilocytosis was observed in 56.9% of cases, and immunostaining for p16 was 49.02%, predominantly in tumors not keratinized (p = 0.03532).

Conclusion
The present study has demonstrated that the infection prevalence of HPV in HNSCC, through the immunostaining with p16, was present in 49.02% of cases. Toward the epidemiological profile, carcinomas were more common in male individuals with middle ages of 61 years and in the larynx as more often topography. Koilocytosis was found in 29 cases, corresponding to 56.86% of our sample. The immunoreactivity of p16 protein predominated in non keratinized tumors.

References


TP53 AND THE ASSOCIATION TO AFATINIB RESPONSE IN HNSCC CELL LINES

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Background / Objectives

The signaling pathway of the epidermal growth factor receptor (EGFR) is commonly activated in HNSCC and represents a target for therapy. Among the different anti-EGFR agents, such as tyrosine kinase inhibitors and monoclonal antibodies (mAbs), the reversible inhibitor Cetuximab is the only approved for HNSCC treatment. However, treatment with these reversible tyrosine kinase inhibitors produces objective responses in only a small subset of patients. Despite an initial positive response, these patients often develop or acquire secondary resistance to the inhibitors, leading to relapse after several months. Thus, the resistance to anti-EGFR reversible inhibitors has emerged as an important clinical problem, making irreversible inhibitors studies essential for HNSCC tumors, such as clinical trials with Afatinib and Allitinib. The aim of this study is to evaluate sensitivity and resistance to anti-EGFR targeted drugs in a panel of HNSCC cell lines (HPV positive and negative), and correlate this profile with genetic alterations.

Methods

Five HNSCC HPV(+) cell lines and five HNSCC HPV(-) cell lines were used to test the treatment efficacy of Cetuximab, Afatinib and Allitinib by cell viability assays (MTS). Cells were seeded in 96 wells plates, exposed to increasing doses of each anti-EGFR inhibitors (0 - 2.5 µM) for 72 hours. For the mutation analysis, a panel of primers covering the entire coding extension of TP53, NOTCH1, P16, PTEN, PIK3CA, FBXW7, HRAS, TP63, CASP8, FAT1, MLL2, RB1, IRF6, NSD1 and EZH2 genes has been customized using AmpliSeq Custom Panel (Life Technologies). The mutational profile of these tumor-related genes was assessed by next-generation sequencing using the Ion Torrent PGM platform.

Results

The results showed that only ten genes (TP53, NOTCH1, PTEN, HRAS, TP63, CASP8, FAT1, MLL2, RB1 and IRF6) had at least one cell line mutated. Several combinations were performed as a panel, where a positive panel was defined as at
least three genes being mutated in the sample, and the result was that 80% of HPV negative cell lines were positive for the panel. Regarding to response profile, all TP53 wild type cell lines were Afatinib sensitive (p=0.033).

**Conclusion**

The “mutational positive” panel related to HPV negative cell lines corroborate with the literature, where HPV negative tumors are more likely to have these genes mutated than HPV positive tumors. Moreover, TP53 mutational status in HNSCC cell lines may predict Afatinib response, showing its feasibility as a potential biomarker in clinical setting.
THE ROLE OF P16 IN THE METASTASIS PROCESS OF HUMAN PAPILLOMAVIRUS POSITIVE HEAD AND NECK CANCERS

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Background / Objectives

Human papillomavirus (HPV) positive head and neck squamous cell cancers (HNSCC) are often characterized by low tumour (T) and high regional node (N) stages, indicating a high lymphatic metastatic potential. Although the dissemination pattern is different, the haematogenous metastatic rate is the same for HPV-positive and HPV-negative HNSCC. The biological mechanism behind this paradoxal dissemination pattern remains largely unknown.

Methods

We assessed the dissemination pattern in HPV-positive HNSCC combining data of 241 patients with in vitro and in vivo models. More specific, the effect of p16 and HPV on metastasis was assessed by invasion and migration assays. In vivo we focussed on angiogenesis (CD31) and lymphangiogenesis (LYVE-1).

Results

Our study cohort confirmed that HPV-positive patients have significantly lower T stages and higher nodal involvement. HPV-positive cells had significantly lower migration rates and invasion capacities compared to HPV-negative cells. Downregulation of p16 increased migration and invasion capacities. To unravel the metastasis process, we focussed on angiogenesis and lymphangiogenesis, which are indispensable for oxygen and nutrient supply for tumour growth. A negative correlation between HPV and VEGFA was seen in the patient cohort and trend to significance was noted between p16 and VEGFA. Suppression of p16 increased the tumour vascularization. Secondly, HPV-positive tumours showed higher number of lymphatic vessels compared to HPV-negative tumours. P16 suppression in HPV-positive models resulted in lower lymphatic vessel density. This can be related to the α4β1 integrin, an important regulator of lymphangiogenesis, as HPV-negative tumours showed high percentages of this integrin.

Conclusion

These results suggest that p16 inhibits growth and invasiveness through inhibition of angiogenesis but also stimulates local spread by lymphatic vessel formation. This offers therapeutic applications for metastasis in HNSCC by inhibiting
lymphangiogenesis in HPV-positive cancers and angiogenesis in HPV-negative cancers.
HUMAN PAPILLOMAVIRUS DNA DETECTION IN FINE-NEEDLE ASPIRATES AS INDICATOR OF HUMAN PAPILLOMAVIRUS-POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: A PROSPECTIVE STUDY.


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Background / Objectives

Human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (SCC) has a better outcome than most head neck squamous cell carcinomas (HNSCCs) and an HPV-positive lymph node metastasis likely has an HPV-positive oropharyngeal SCC origin. Determining HPV-status in cervical lymph nodes by fine-needle aspiration cytology (FNAC) may be useful for diagnosis.

Methods

FNACs from 66 patients with neck masses were prospectively examined for HPV DNA and HPV16 mRNA by a polymerase chain reaction (PCR)-based assay, and the data correlated to diagnosis and HPV-status obtained from histopathological specimens.

Results

Aspirates from 17 of 66 patients, later diagnosed with HPV-positive oropharyngeal SCC, were HPV16 DNA-positive. HPV16 mRNA was detected in all cases with extractable RNA. All remaining FNACs, including 18 branchial cleft cysts, were HPV DNA-negative. HPV DNA status in the aspirates showed perfect concordance with corresponding biopsies.

Conclusion

HPV16 DNA detection in fine-needle aspirations from neck masses is reliable and HPV16 DNA in a metastasis is a strong indicator of an HPV-positive oropharyngeal SCC.
THE COUPLE MANAGEMENT OF HPV INFECTION

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Background / Objectives

Human papilloma virus (HPV) is considered a worldwide public health problem, with 70% of cervical cancers being incriminated to strains 16 & 18 and 90% of genital warts on 6 & 11. With recent tools of molecular biology, we now know there are over 200 genotypes, with very different anatomical site tropism. We aim to demonstrate the implication of HPV in tongue cancer carcinogenesis and the importance of couple diagnosing.

Methods

We present the case of a 27 years old woman who was diagnosed with tongue cancer T2N1M0, in which by PCR reverse hybridization on the biopsy sample HPV 16 strain infection was identified. The patient followed standard surgical and oncological protocol for tongue neoplasm, with favourable outcome, with no signs of local recurrence at two years.

At the same time, she was colposcopically investigated, thus detecting moderate cervical dysplasia (CIN 2), while cervical HPV typing identified the same 16 strain infection. The cervical lesion was surgically treated by diathermal loop electroresection, and the HPV retesting at 3 months post-intervention was negative. The patient received the anti-HPV tetravalent vaccine, using the complete protocol.

The partner, age 41, was diagnosed in a dermato-venerology service with penile warts. HPV genotyping identified the strains 16, 31 and 40 as positive. Penile lesions were cauterized and the patient was vaccinated anti-HPV on request with complete protocol without local recurrence at two years.

Conclusion

Interdisciplinary collaboration, consisting in a medical team of otorhinolaryngologists, oromaxilofacial surgeons, gynaecologists, urologists and dermatologists, contributed to the optimal management of this case of couple HPV infection.

The detection of one or more strains of HPV in the genital or otolaryngology area in one of the partners of a couple requires a complete medical history and physical examination.
investigations for the detection of all possible HPV-induced lesions regardless of their location in both partners.
The prognostic utility of HPV specific testing in addition to p16 immunohistochemistry in oropharyngeal carcinoma.

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Background / Objectives

Patients with oropharyngeal squamous cell carcinoma (OpSCC) have significantly improved overall survival (OS) compared to matched HPV negative control groups. This observation has led to several ongoing clinical trials evaluating deintensification treatment strategies in HPV positive disease. Current guidance from UK national and professional organisations recommend p16 immunohistochemistry (IHC) as a minimum test which should ideally be followed by HPV-specific testing with DNA in situ hybridisation (ISH) in p16 positive cases. 1,2 However, most UK diagnostic and treatment centres limit HPV testing to p16 IHC alone. Furthermore, several clinical trial protocols randomise patients according to p16 status alone without necessitating HPV specific testing. The objectives of this study were to:

1. Determine the prevalence of p16 positive tumours lacking high-risk HPV DNA by ISH (p16+/DNA ISH-) in OpSCC.

2. Compare OS of patients with p16 positive tumours demonstrating high-risk HPV DNA ISH positivity (p16+/DNA ISH+), p16+/DNA ISH- and p16 negative (p16-) OpSCC.

3. Determine whether p16+/DNA ISH- can further be classified using RNA ISH.

Methods

Consecutive OpSCC cases from two large UK treatment centres were tested for p16 IHC. High-risk HPV DNA ISH was undertaken on all p16 positive tumours. RNA ISH was performed on a subset of p16+/DNA ISH- cases. OS was determined from patient records and evaluated using SPSS software.

Results

There were 347 patients included in this study. The prevalence of p16+/DNA ISH- was 11.8%. Patients with p16+/ISH- OpSCC had poorer OS compared with p16+/DNA ISH+ tumours (mean OS 45.1 vs 53.2 months, p=0.001), but improved OS compared with p16- tumours (mean OS 45.1 vs 33.7 months, p=0.023). Twenty-four p16+/ISH- samples were available for RNA ISH testing, of which 15 were finally classified as HPV positive and 9 as HPV negative.

Conclusion
Patients with p16+/DNA ISH- OpSCC have poorer OS compared to those with p16+/DNA ISH+ tumours, but improved OS compared with p16- tumours. Therefore, for purposes of clinical trial stratification and prognostication, we recommend HPV DNA ISH testing on all p16+ cases. RNA ISH is a suitable test for resolving the HPV status in p16+/DNA ISH- tumours.

References

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P27-12
FREQUENCY AND CLINICAL OUTCOME OF HPV-DRIVEN OROPHARYNGEAL CARCINOMA IN NORTH-EAST ITALY

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Background / Objectives

HPV-driven oropharyngeal carcinoma (OPSCC) is on the rise in many European countries. Our objective is the assessment of frequency over time of HPV-driven OPSCC in our area and the clinical outcome in comparison to HPV-unrelated cases.

Methods

Fresh/frozen or formalin-fixed paraffin-embedded tumor specimens obtained at OPSCC diagnosis from consecutive patients (period evaluated 2003-2016) were analyzed by PCR with MY09/MY11 primers and restriction fragment length polymorphism analysis for search and typing of HPV-DNA sequences; HPV16 viral load (E6 copies/cell) was determined by real-time PCR; p16 expression was evaluated by immunohistochemistry. Presence of HPV-DNA, HPV16 viral load >1 E6 copy/cell, and p16 overexpression defined an OPSCC as HPV-driven. Frequency of HPV-driven OPSCC was calculated overall and by time periods (2003-2007; 2008-2012; 2013-2016). Overall Survival (OS) and Progression Free Survival (PFS) were calculated by Kaplan-Meier method and Cox-regression and compared among patients with HPV-driven and HPV-unrelated OPSCC.

Results

Overall, 101 cases of newly diagnosed OPSCC were included; data on 63 of them have been previously published (1-3). HPV-DNA sequences were detected in 31 of them; HPV16 was present in 29, and HPV58 and HPV33 in 1 case each. Up to now, the causal role of HPV has been proven in 28 cases (26 HPV16, 1 HPV58 and 1 HPV33). The prevalence of HPV-driven OPSCC in the three time periods was 17.4% (4/23), 25.6% (10/39) and 35.9% (14/39), respectively (P=.109). Patients with HPV-driven tumors had both improved OS (HR = 0.25, 95% CI = 0.10 to 0.62; P=.003) and PFS (HR = 0.23, 95% CI = 0.10 to 0.55; P=.001).

Conclusion

The frequency of HPV-driven OPSCC is on the rise also in North-East Italy, an area with known low prevalence. Our data confirm the good prognosis of patients with HPV-driven OPSCC.
References


P27-13
PREVALENCE OF HPV IN BRANCHIAL CLEFT CYSTS AND THE USE OF HPV IN DIAGNOSIS OF CYSTIC LESIONS OF THE NECK

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Background / Objectives

The difficulty to distinguish a branchial cleft cyst from a cystic metastasis is well known. Branchial cleft cyst is a benign condition which is treated by surgery with relatively low morbidity. Cancer of unknown primary has formerly been treated with neck dissection and then radiotherapy. We, like many head and neck cancer centers in the world, has in recent years relied on the cytological diagnosis of SCC (which can be hard) in combination with HPV analysis, and if the metastasis is HPV positive it is assumed that the primary tumor is in the base of tongue or the tonsils. Then the patient receives radiotherapy. The problem is that this shift in diagnostic and treatment has occurred, even internationally, without anyone really examined whether HPV can also occur in branchial cleft cysts. The risk is that if we rely on HPV assay as a diagnostic tool for cancer, even if branchial cleft cysts are found to be harboring HPV, patients with HPV positive branchial cleft cyst can receive unnecessary cancer treatment.

In a previous prospective study of FNAC in neck masses we found 18 branchial cysts which all were HPV-negative.1

Methods

All patients over 18 years who underwent surgery for branchial cleft cysts from 2005 to 2015, about 400 pc, is included. HPV assay is performed with Multiplex Luminex HPV PCR method in histological sections from paraffin-embedded material from 2005-2015 from the cyst surgically removed.

Results

12 branchial cleft cysts have so far been analyzed, and they have all been HPV-negative.

Conclusion

Reliable methods for diagnosis of cystic lesions the neck is essential for proper diagnosis and treatment. There is, so far, not any published articles regarding HPV in branchial cleft cysts.

References
EXPRESSED HPV INTEGRATION EVENTS IN HEAD AND NECK CANCER: WHERE THEY OCCUR AND THEIR EFFECT ON SURVIVAL AND MOLECULAR SIGNATURES

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Background / Objectives

The incidence of human papillomavirus (HPV)-related oropharyngeal cancer has risen to epidemic levels in the United States. One common molecular event during HPV-related oncogenesis is full or partial integration of the HPV genome into the host genome. Expression of the integrated HPV early genes has clinically-relevant effects. However, the specific nature of where these integration events occur, their effect on patient survival, and the extent to which the switch to integrated HPV integration affects differentiation, the host immune response, and DNA copy number changes is unknown. Previously, our group characterized two HPV-related tumor subtypes, finding that they differed by HPV integration status, immune response, keratinization, EMT signatures, type and number of DNA copy number alterations, and PIK3CA mutations. Interestingly, many of the differences could be explained by HPV integration.

Methods

In this study, we identified and characterized expressed HPV integration events from 84 HPV-positive oral cavity and oropharyngeal tumors (18 from the University of Michigan Hospital (UMH) and 66 from The Cancer Genome Atlas (TCGA)). Flash frozen tumor tissue was collected at UMH for 36 oral cavity and oropharyngeal cases, and H&E slides were assessed for degree of cellularity (minimum 70%) and necrosis (less than 10%). Upon mRNA-seq using 100nt paired-end reads on an Illumina HiSeq, half (18/36) were identified as HPV-positive. HPV integration events were detected using RNA-seq data, captured by HPV-host fusions transcripts. Virus-seq was used to detect HPV-host fusion events, the edgeR R package was used to determine differential expression, and a Cox proportional hazards model was used for survival analysis.

Results

We found 320 virus-host integration breakpoints in 51 (61%) of the 84 samples. Integration events were strongly overrepresented near known head and neck, lung, and urogenital cancer genes, with five recurrent genes (including PD-L1). They were enriched in certain classes of repetitive regions, and a significant number of genes harboring an integration were found to interact with Tp63, ETS, or FOX1A. Patients
with no detected integration had significantly better survival than those with a detected integration and HPV-negative patients.

**Conclusion**

Our results suggest that in HPV-related tumors of the upper aerodigestive tract, there is strong natural selection for cells with expressed HPV integration events in/near key oncogenes and tumor suppressors. The survival benefit of those patients having no expressed integration event provides a candidate cohort for de-escalated therapy.

**References**

E6 PROTEINS OF ALPHA AND BETA CUTANEOUS HPV TYPES DIFFER IN THEIR ABILITY TO POTENTIATE WNT SIGNALING

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Background / Objectives

HPV types which belong to the beta-PV genus have been implicated in the development of non-melanoma skin cancer (NMSC). Our recent studies found that the E6 protein of HPV16, a mucosal high risk HPV type from the alpha genus, is capable to cooperate with the ubiquitin ligase E6AP to enhance or stimulate Wnt/beta-catenin/TCF transcription. In the present study we investigated the transcriptional activities of E6 proteins of diverse HPV types that infect the skin, both from the beta and alpha HPV genus.

Methods

Luciferase reporter gene assays, Western blots, immunoprecipitation and immunofluorescence analyses

Results

Using reporter gene assays, we show that similar to 16E6, E6 of HPV10, an alpha HPV type which is prevalent in skin warts, is capable to efficiently augment as well as stimulate Wnt/beta-catenin/TCF transcription. Western blot and immunofluorescence analyses indicated that 10E6 also elevated efficiently the expression levels of beta-catenin and promoted its nuclear accumulation. E6 proteins of the beta HPV genus including HPV 8, 24, 38 and 49, exhibited lower activities in enhancement or stimulation of beta-catenin/TCF transcription, as well as reduced ability to stabilize β-catenin. The difference in levels of activity between the alpha and beta HPV E6 proteins correlated with the ability of the proteins to interact with E6AP.

Conclusion

This study revealed a role for E6 proteins of diverse skin associated HPV types in potentiating Wnt/beta-catenin/TCF signaling irrespective with their carcinogenic potential.
LOW VACCINE COVERAGE BUT NEAR EXTINCTION OF HPV 6 AND GENITAL WARTS IN YOUNG WOMEN IN WOLFSBURG, GERMANY

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Background / Objectives

It has been shown that vaccination of HPV naive women against HPV 6/11 protects sufficiently from genital warts and may even lead to protection of non-vaccinated men and women in the same population. However it is uncertain what level of vaccine coverage is needed for such cohort effects.

Methods

WOLVES (Wolfsburg HPV epidemiological study) invited all women born 1983/84 and 1988/89 with a first residency in Wolfsburg to participate in 2009/10. Participants born 1988/89 were followed with annual examinations from 2009/10 till 2014/15. Women born 1993/94 were first invited 2014/15 and are followed with annual visits. HPV-testing is based on LR and HR-HC2 followed by HPV genotyping with SPF-10 PCR of all HC2 positive and 10% of HC2 negative samples.

Results

Between Oct 2009 and Dec 2015, 2,360 women were recruited. The HPV vaccination coverage rate rose from 6.1% among 26 years old women in 2010 to 18.4% in 2015 while the corresponding rates for 21 years old women increased from 23.7 to 48.2%. Simultaneously the life risk to suffer from at least one episode of genital warts before age 27 dropped from 4.7% in 2010 to 2.5% in 2015 while the life risk before age 22 declined from 1.8% to 0.4%. This trend of disappearance of genital warts was underlined by a decline in the prevalence of HPV 6 from 2.1% to 0.2% among 26 years old women between 2010 and 2015 and from 2.0% to 0.0% among 21 years old women.

Conclusion

The unexpected significant drop in genital warts and HPV 6 prevalence in a population with suboptimal HPV vaccine coverage is reassuring. Obviously the transmission of HPV as the causal agent of most genital warts is strongly inhibited even in populations with low vaccination coverage.
ACCURATE AND SPECIFIC DETECTION OF 13 GENOTYPES ASSOCIATED WITH SEXUALLY TRANSMITTED DISEASE BY PNA MEDIATED REAL TIME PCR

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Background / Objectives

Sexually transmitted diseases (STD), also referred to as venereal diseases (VD) are diseases that can be spread by sexual activity or sexual contactless infection such as transmission via infected blood. Some of STD pathogens such as *Gardnerella vaginalis* (GV) show no symptoms in men but has the ability to infect women. Other pathogens such as *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) may appear chronic pain, itching, small fluid-filled blisters and painful sexual intercourse or even infertility in women and it can be prescribed antibiotics. *Neisseria gonorrhoea* (NG) causes symptoms of cervicitis, urethritis and others in women and men both. NG or CT can be transmitted from a pregnant mother to unborn child. Therefore, detection of STD pathogens is essential for accurate treatment because each type of STD pathogens requires different prescription of antibiotics.

Methods

We have developed a highly sensitive and simple real-time PCR method to detect 13 types of STD pathogen from DNA in patient’s specimens such as cervical swab and urine both for female and male. This method (PANA RealTyper™) uses specific peptide nucleic acid (PNA) probe conjugated with a fluorescent dye and a quencher. These PNA probes are designed to their specific target sequences, which results in each having unique Tm value.

Results

Test was performed to obtain 40 cervical swab samples and 35 urine samples. DNA was extracted from 40 cervical swab samples and 35 urine samples. PANA RealTyper™ STD analysis showed a concordance rate of 92% in 69 samples of 75 samples. Mismatched 6 samples were confirmed via sequencing, it showed that the result of PANA RealTyper™ STD matched sequencing PCR. 40 of 85 clinical specimens were infected samples with two or more STD pathogens.

Conclusion

RealTyper™ STD was able to detect multiple pathogen targets at the same time. PANA RealTyper™ STD is able to detect 13 types of different pathogens with detection limits as low as 5x10^1 copies/rxn using standard materials. It provides genotyping of a total 13 types (*Chlamydia trachomatis, Trichomonas vaginalis,*...
Ureaplasma pavum, Ureaplasma urealyticum, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Haemophilus ducreyi, Herpes Simplex Virus 1, 2, Gardnerella Vaginalis, Candida albicans, Treponema pallidum) by real-time PCR.
HPV infection among HIV-positive men: A four year revised experience of an diagnosis Laboratory.

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Background / Objectives

The spread of HIV epidemics globally has increasingly drawn attention to the interaction between HIV and the “classic” sexually transmitted infections (STIs). A consensus has grown that other STIs increase the spread of HIV, following on from the early epidemiologic studies that explored the epidemiologic synergy between STIs and HIV.

However, the interaction of the many STIs with HIV is potentially complex, with the possibility of reciprocal influences on susceptibility, infectiousness, and the natural history of infections.

There is growing evidence of a significant burden of human papillomavirus (HPV) infection and associated disease in men.

HIV infection increases HPV prevalence, incidence and persistence and is strongly associated with the development of anogenital warts as well as anal, penile, head and neck cancers in men. Despite increasing access to antiretroviral therapy, there appears to be little benefit in preventing the development of these cancers in HIV-positive men, making prevention of infection by vaccination and information, a priority.

The authors present a 4 years revised casuistic as a reference laboratory center in sexually transmitted infection diseases diagnosis.

Methods

Male samples were tested by HPV-molecular and conventional-cytology methods. HPV molecular methods used where: Hybrid Capture 2 (hc2, Digene) ; Clart human papillomavirus 2 (Genomica) and PapilloCheck. The cytological results were registered with comprehensive classification system, multi-axial nomenclature SNOMED. The diagnosis of “classic” sexually transmitted infections (STIs) as Herpes Simplex virus 1 and 2, Syphilis, Gonorrhea, Chlamydia trachomatis, Ureaplasma and Mycoplasma infections statistics were used for data analysis; the Fisher exact test was employed to assess the association between categorical variables. P-values (2-sided test) less than 0.05 were considered significant.

Conclusion
The results obtained for the incidence of most frequent HPV genotypes in men and MSM are in agreement with several studies. Type 16 was consistently found among the most common; however, other types were also reported (types 6, 11, 18, 31, 33, 42, 52, 53, 54, 59, and 84) but a shift possibility can occur with universalization of the vaccine. HPV infection appears to occur early in MSM. The majority of MSM followed in Proctology consult first diagnosed for anal or perianal condyloma was offered starting HPV vaccination. It will be an interesting development of this work, following up some of these patients and document relapsing and the HPV genotypes evolved after complete vaccination schemes.
P32-01
ESTIMATING THE EPIDEMIOLOGICAL IMPACT AND COST EFFECTIVENESS OF THE NEW NONAVALENT HPV VACCINE IN SPAIN

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Background / Objectives

Human papillomavirus (HPV) is one of the most frequent sexually transmitted infection and one of the main causes of infection-related cancer, accounting for 5% of the total burden of human cancer worldwide. Since 2007, two vaccines were available in Spain: the quadrivalent HPV vaccine (HPV4), that contains types 6/11/16/18 and the bivalent HPV vaccine (HPV2), that contains types 16/18. It is estimated that 6/11/16/18 are responsible of 47% of precancerous anogenital lesions due to HPV, and 79% of cancers related to HPV. A nonavalent vaccine (HPV9) containing HPV types 6/11/16/18/31/33/45/52/58 has been developed. According to epidemiological data, the 9 types are responsible of 82% precancerous lesions and 90% of cancers related to HPV.

The aim of this project is to assess the epidemiological impact and the cost effectiveness of 9vVPH in Spain.

Methods

We adapted to the Spanish setting an integrated HPV disease transmission model that accounts for herd protection effects and with a 100-year time horizon.

Results

The model shows further reductions in the incidence and mortality of diseases related to HPV16/18/31/33/45/52/58 types over the analyzed time horizon with HPV9 vaccination when compared to HPV4 or HPV2 in both girls-only and universal vaccination scenarios. 9vVPH would avoid additional 161,485 CIN1 cases, 112,393 CIN2/3 cases, and 15,380 cervical cancer cases compared to HPV4 and HPV2 in a girls only vaccination scenario. With regards to genital warts, 1,466,379 and 1,395,111 cases, in females and males respectively, would be prevented by HPV9 versus HPV2 in a girls only vaccination and 1,629,959 cases in females and 2,027,372 cases in males, in a universal vaccination setting.

Girls-only vaccination strategy of a 12 years old cohort with HPV9 was found to be cost effective compared to HPV4 (ICER of €14,000/QALY) and a dominant strategy compared to bivalent HPV vaccine (HPV2). In a universal vaccination scenario,
HPV9 is cost-effective compared to HPV2 (ICER of €3,716/QALY) and is cost-effective vs. HPV4 Girls if HPV9 price does not exceed €121 per dose.

Conclusion

A significant reduction in the HPV-related disease is expected after HPV9 introduction. In addition, HPV9 vaccination is cost-effective when compared to the current HPV4 and HPV2 vaccination programs. Moreover, the inclusion of boys in the HPV9 vaccination program could potentially further reduces the burden of HPV-related diseases in both genders in Spain.
GARDASIL9: ACCELERATED REDUCTION IN THE INCIDENCE AND COSTS OF HPV-RELATED PRECANCEROUS LESIONS AND CANCERS

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Background / Objectives

Gardasil9 – protecting against HPV 6, 11, 16, 18, 31, 33, 45, 52 & 58 - is available and indicated in males and females to protect against

- Premalignant lesions and cancers affecting the cervix, vulva, vagina and anus caused by vaccine;

- Genital warts (Condyloma acuminata) caused by specific HPV types (1).

Compared to Gardasil and Cervarix, Gardasil9 improves the protection against precancerous lesions and cervical, vulva, vaginal and anal cancers (2) implying that Gardasil9 lead to an accelerated reduction in the incidence of precancerous lesions in the cervix, vulva and vagina and anus and in the incidence of cervical, vulva, vaginal and anal cancers. Consequently, the future saved costs of treatment of precancerous lesions and costs of treatment of cancer will be reduced even more.

In this study, the extra costs saved in Denmark using Gardasil9, compared to Cervarix and Gardasil, in the Danish HPV-vaccination programme targeted girls will be estimated.

Methods

The analyses are based on previous published model simulations and updated unit cost estimates (3). In addition, the following limitations and assumptions are made:

- It is assumed that Gardasil and Cervarix has the same relative protection against CIN2+ (cervical intra-epithelial neoplasia), cervical, vulva and vaginal cancers;

- Since Gardasil9’s extra protective effect against anal cancer is little, and since unvaccinated men (and ignoring the herd immunity protection) also are diagnosed with anal cancer, this extra effect is ignored in the calculations; and

- Since no Danish unit cost estimates for the precancerous lesions in the vulva or vagina and anus (VIN 2/3, VaIN 2/3 and AIN 2/3) are published/available, Gardasil9’s extra protective effect against VIN 2/3, VaIN 2/3 and AIN 2/3 is also ignored.

Results
Compared to Cervarix and Gardasil, the extra costs saved given Gardasil9 vaccination is estimated to 3.2 mill. € (PV: present value) per vaccinated cohort. Especially the additional reduced incidence of CIN2+ and cervical cancer lead to sizeable extra costs saved – 2.5 mill. € (PV) and 0.65 mill. € (PV), respectively.

In addition, Gardasil and Gardasil9’s protection genital warts lead to extra saved treatment costs compared to Cervarix.

Conclusion

In a Danish setting, a Gardasil9 vaccination programme will lead to an increased reduction in the incidence and costs of HPV-related precancerous lesions and cancers.

References

P32-03
COST-EFFECTIVENESS OF CERVICAL CANCER SCREENING IN EUROPE – A SYSTEMATIC REVIEW WITH SPECIFIC INTEREST ON RISK-ADAPTED STRATEGIES

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Background / Objectives

In Europe, cervical cancer screening varies substantially regarding age at screening start and end, frequency, type of primary test and follow-up algorithm for screen-positive women. Risk-based screening and follow-up strategies may have the potential to improve both, the benefit-harm balance of the screening program and its cost-effectiveness. We systematically reviewed current evidence on long-term effectiveness and cost-effectiveness of cervical cancer screening in Europe with specific interest on risk-adapted strategies.

Methods

Relevant databases (Medline/Embase/Cochrane Library/CRD/EconLit) were systematically searched for decision-analytic studies evaluating the cost-effectiveness of cervical cancer screening strategies in Europe. Study characteristics and results including the incremental cost-effectiveness ratios (ICER) in cost per quality-adjusted life year (QALY) or life year gained (LYG) were extracted into standardized evidence tables. Economic results were converted to 2015 Euros.

Results

Fourteen studies were included, comprising eleven analyses for countries with population-based organized screening, one for an opportunistic screening, and two for countries where, depending on the region, the screening is organized or opportunistic. Three studies evaluated screening for multiple European countries.

HPV-based screening was reported to be more effective in terms of patient-relevant outcomes compared to cytology alone in both non-vaccinated and vaccinated
women. Overall, HPV-based screening strategies were considered to be cost-effective at a willingness-to-pay threshold of 50,000 Euro/QALY or LYG conditional on screening intervals of at least three years in non-vaccinated women and at least five years in vaccinated women. Most studies recommended starting screening at age 25 with HPV-based screening at age 30 years or older. HPV screening was mostly accompanied with a triage test for HPV-positive women. The upper age limit for screening varied with most studies ending screening at age 65 years.

Conclusion

In conclusion, the evidence from decision-analytic modeling studies suggests that HPV-based screening is more effective compared to cytology alone, and can be considered cost-effective at screening intervals of at least 3 years in non-vaccinated and at least 5 years in vaccinated women. Current risk-tailored screening programs are based on restrictions to a specific age and using triage or follow-up tests for HPV-positive women before referring to colposcopy directed biopsy. In future research, predictive biomarker for risk-based management of screen-positives should be considered.
A SURVEY OF HPV KNOWLEDGE AND ATTITUDES TOWARD HPV VACCINATION AMONG THE MIDDLE SCHOOL STUDENTS AND THEIR PARENTS IN CHINA*

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Background / Objectives
Cervical cancer is one of the most common cancers afflicting women worldwide. The wide disparities in the incidence and mortality of cervical cancer are mainly attributable to irregular education and economic development in China. We assessed the knowledge of HPV and attitudes towards HPV vaccination among middle school students and their parents in Changzhi city of north China from June 2015 to March 2017, in order to provide an evidence for the development of an effective national HPV vaccination program.

Methods
We selected 6 middle schools from urban and rural area in Changzhi city of north China. 2 first-grade classes were randomly choose from each school as the intervention group (given education related to knowledge of cervical cancer and HPV vaccination), and other first-grade classes in the same schools as the control group. The health education was conducted by the qualified teacher for intervention group. The questionnaire related the knowledge on cervical cancer was conducted before and after class. The same test was performed in the control group. The two groups were followed up to evaluate the effect of education in 2016 and 2017, respectively. Their parents of the students were required to accept the education of cervical cancer and HPV vaccination, and complete the questionnaire before and after the lecture.

Results
1241 students, aged from 11-15 years, from 6 middle schools attended the study in the baseline survey, 568 in intervention group and 673 in control group, respectively. We found only 34.89% of participates heard of cervical cancer, 10.87% heard of HPV, 7.17% heard of HPV vaccine. After intervention, there were a significantly improvement the awareness about cervical cancer and HPV vaccine (P<0.05). 91.02% of participates would like to HPV vaccine (P<0.01). There were a significant difference between intervention and control group (P<0.01). Comparing the follow-up results of survey in 2016 and 2017, we found there still were a significant with the awareness between intervention and control group (P<0.05). In the baseline, we found that 29.87% of 233 parents heard of the HPV, and 20.34% heard of the HPV vaccine in the survey. Also, there were a significantly improvement the awareness about cervical cancer and HPV vaccine (P<0.05) after the health education.

Conclusion
The level of knowledge on cervical cancer and HPV vaccine was lower in the subjects. We should strength public and middle schools students education with regard to cervical cancer and HPV vaccination to support increased uptake of cervical cancer prevention and control in China. Also it is necessary to shorten the interval between health educations in order to improve the effect of it.

References

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HPV – WHAT DO THEY KNOW: EVALUATION OF MEDICINE STUDENTS AND HEALTH PROFESSIONALS – VALENÇA – RIO DE JANEIRO/BRAZIL

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Background / Objectives

One of the most prevalent sexually transmitted diseases affecting the population is Human Papillomavirus (HPV), which is one of the most common infections among young and sexually active individuals, in which 75-80% of the population has been, is or will be infected during his life. The lack of information about the Human Papilloma Virus, the signs and symptoms of the infection, its relation to cervical cancer and the forms of transmission, contributes to the female sex being more exposed to this disease. Thus, there is a need to research the understanding of the students and health professionals of an educational institution regarding HPV, since information is the main basis of health prevention. Objective: To analyze the knowledge of medical and non-medical professionals, medical students and students of other courses regarding HPV (Human Papilloma virus).

Methods

We interviewed 269 people in the city of Valença - RJ, with an identification questionnaire and questions about HPV. Among those interviewed are: doctors, medical students, non-medical professionals and students from other courses of the Dom André Arcoverde Foundation - FAA.

Results

The results were divided into 4 variables, such as: interviewee profile, knowledge of health professionals, high school students and non-medical professionals about HPV, relationship with uterine cancer and respect for the vaccine, questioning about acceptance of the vaccine against HPV.

Conclusion

The results of the study indicate that the majority of health interviewees have knowledge about the Human Papilloma virus, its etiology, as well as its form of transmission and prevention. On the other hand, it is noticed that there is still a lack of knowledge on the part of the staffs and students of other courses.

References


Well-Child Visits and HPV Vaccination in Preteenagers Aged 11-12 Years during 2007-2015 in the United States

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Background / Objectives

Well-child visits (WV) provide the best opportunities for vaccinations in the US. The Advisory Committee on Immunization Practices (ACIP) recommends vaccination of 11-12 year old females and males with HPV (Human Papillomavirus) vaccine. This study aims to estimate the % of preteenagers aged 11-12 years who had WV and the % received the first dose of HPV vaccine (HPV1) from 2007 to 2015 and to estimate, for those preteenagers who did not receive HPV1, the % of them had a WV in the future.

Methods

This was a retrospective database (MarketScan®) cohort study. Eligible subjects were 11-12 year old females and males who had continuous enrollment since January 1, 2007 or January 1 of the year they turned 9 years old and did not have HPV vaccine previously. Females were excluded if they had a medical claim related to pregnancy, delivery, cervical cancer, or hysterectomy. Percentages of WV and HPV1, overall and during WV, were estimated.

Results

There were a total of 1,922,372 eligible subjects; 58.8% 11 years old vs. 41.2% 12 years old and 78.2% females vs. 21.8% males. From 2007-2015, 51.3% of 11 year olds and 53.4% of 12 year olds had WV; 9.8% of 11 year olds and 14.0% of 12 year olds initiated HPV1. Among those who initiated HPV1, approximately 53% were during WV for both 11 and 12 year olds. For those who did not receive HPV1 at 11-12 years old, less likely they would have a WV in the future, ranging from 36.6% at 12-13 years old to 5.2% at 19-20 years old.

Conclusion

This analysis suggests that WV at 11-12 years of age provide the best opportunity to maximize the potential of the HPV vaccination program in the US.
P36-02
ACCEPTABILITY AND SHORT-TERM EFFECTS OF AN HPV VACCINATION INTERVENTION FOR YOUNG GAY AND BISEXUAL MEN IN THE UNITED STATES

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Background / Objectives

Gay and bisexual men have high rates of human papillomavirus (HPV) infection and HPV-related disease, such as anal cancer. Despite these existing disparities, no known interventions have been developed to increase HPV vaccination among young gay and bisexual men (YGBM) in the United States. We developed and pilot tested an online intervention, Outsmart HPV, to promote HPV vaccination among YGBM.

Methods

In 2016, we used social media to recruit a national sample of YGBM ages 18-25 in the United States who had not received any does of HPV vaccine (n=150). Participants were randomized to receive either standard information about HPV vaccination (control group) or population-targeted, individually-tailored content about HPV vaccination (intervention group). Participants in both study arms completed a baseline survey before viewing their study materials and a follow-up survey immediately afterwards. We used multiple linear regression to assess between-group differences in attitudes and beliefs about HPV vaccination.

Results

Most participants were ages 22-25 (59%), gay (83%), non-Hispanic white (57%), and not married or living with a partner (80%). There were no differences in HPV vaccination attitudes and beliefs between study arms at baseline. Compared to participants in the control group, participants in the intervention group reported on their follow-up surveys: greater perception of increased risk for anal cancer among men who have sex with men compared to other men (b=0.30), fewer perceived harms of HPV vaccine (b=-0.34), and greater self-efficacy to get HPV vaccine (b=0.18) (all p<0.05). Results also suggest a trend toward higher intent to get HPV vaccine among participants in the intervention group compared to those in the control group (b=0.21, p=0.09). Participants in the intervention group reported high levels of acceptability and satisfaction with their viewed study materials about HPV vaccination (all means >4.40 on a 5-point scale). Further, participants in the intervention group more strongly endorsed that their materials were easy to understand than did the control group (means: 4.72 vs. 4.42, p<0.05).

Conclusion
Findings from this pilot randomized controlled trial provide preliminary support for an online HPV vaccination intervention being highly acceptable to YGBM and improving their attitudes and beliefs about HPV vaccination. Taken together, these results suggest that Outsmart HPV may be a promising tool for promoting HPV vaccination among YGBM. An important next step is to determine the efficacy of the intervention on increasing HPV vaccine uptake among this population.
Background / Objectives

In the U.S., uptake of HPV vaccine remains significantly behind the Healthy People 2020 goal of 80% series completion. While some countries have implemented very successful HPV immunization programs, others have encountered significant political, policy, and logistical barriers. In the U.S., the policy, implementation, and adoption of HPV vaccine has been particularly complicated. As with many other medical innovations, diffusion and adoption is not always rapid and depends on a variety of social and cultural factors, as well as the nature of the innovation itself. Research indicates there is a great deal of 1) confusion and uncertainty about HPV vaccine and 2) concomitant misinformation about the HPV vaccine, who it is meant for, and the conditions under which it is maximally effective. The objective of our study was to develop and evaluate a web-based approach to encourage HPV vaccination in New Mexico, an ethnically diverse U.S. state.

Methods

With funding from National Institute of Allergy and Infectious Diseases, our team conducted a project to systematically develop a set of web-based tools to prompt the informed adoption of HPV vaccination. We used Diffusion of Innovations Theory and related research on Informed Decision Making to guide the iterative development of a website for parents of young female adolescents.

Results

Our presentation will review the website (GoHealthyGirls.org) and present development and preliminary efficacy data from the study. Subsequent funding from the Patient Centered Outcomes Research Institute (PCORI) and the National Cancer Institute (NCI) has provided the opportunity to translate the GoHealthyGirls website to a mobile device responsive format (mobile web app: Vacteens) for parents and girls and to develop a parallel web app intervention for young adolescent boys and their parents. Both new projects will involve large cluster randomized controlled efficacy trials with parents and their adolescent children in New Mexico clinics to determine the impact of these mobile web apps on informed decision making and uptake for the HPV vaccine.

Conclusion

This presentation will discuss the progress and initial results of these ongoing research efforts and the implications for reaching HPV vaccine uptake goals set by Healthy People 2020 in the United States. The presentation will focus on how web-
based interventions show promise for reaching HPV vaccine uptake goals. A mobile web app can make decision-making tools widely available on popular mobile platforms such as tablet computers and smartphones as well as personal computers.
P36-04
Croatian pathway in implementing HPV vaccination (2007.-2017.)

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Background / Objectives

Cervical cancer is one of the most common types of cancer in Croatia, especially among women between 20 and 49 years of age. Sixty five percent of all STIs occur in population under 25 years of age. HPV infection is the most common viral STI. In 2015, 111 cervical cancer related deaths were reported, meaning one woman every third day. These numbers alert for sexual education, HPV vaccination and cervical cancer screening.

Methods

The aim of this study is to present ten year pathway in implementing HPV vaccination in Croatian National Immunization Programme as voluntary and free of charge

Results

The HPV vaccine has been registered in Croatia since 2007. The first recommendation for vaccination against HPV was published by Croatian Society for Gynecology and Obstetrics, for Cervical Cancer, Urogenital and STD and Dermatovenerology and Society for School and University Medicine. First HPV vaccinations were introduced in Zagreb, capitol city of Croatia.

In 2008 HPV vaccination highlighting the importance of school based health education on vaccine availability among pupils and their parents. Financed by local self-government, partially or totally, HPV vaccination becomes available in other parts of Croatia.

In 2009 Professional Associations of the Ministry of Health for the Prevention of Cervical Cancer and Other HPV Vulnerabilities was established and adopted guidelines for introducing the vaccine into the national vaccination schedule.

In 2010 Ministry of Health recommends vaccination against HPV to reduce the risk from HPV infection and the development of cervical cancer.
In 2015, the National Immunization Program was interpolated free of charge optional vaccination for 8th grade female students.

In 2016, the Ministry of Health declares free of charge optional vaccination for male and female students in the 8th grade of primary school and 1st grade of high school, as well as catch up possibility.

From 2017, since HPV vaccination was registered in Croatia as well as on the impleducation of pupils and their parents through parental meetings in schools, information through polyvalent consultation centers and additional school notifications (posters, info sheets) were provided. Average vaccine coverage varied between 5 and 15%.

**Conclusion**

All professional and scientific follow-ups show that awareness of personal responsibility and sufficient information are protective factors in young people’s sexual behavior. HPV vaccination proved to be highly efficient in cervical cancer prevention. However, low response to HPV vaccine in Croatia indicates necessity for revising existing public health programs.

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HPV prevalence and associated risk factors in women with cervical pre-cancer and cancer in Switzerland at the beginning of the cantonal vaccination programmes: The CIN3+plus study

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Background / Objectives

The Swiss Federal Office of Public Health has recommended vaccination against human papillomavirus (HPV) to prevent cervical cancer since 2007. To monitor the future public health impact of vaccination, baseline population-based data are required. The objectives of this study were to determine the prevalence of HPV and examine associated risk factors in women with cervical intraepithelial neoplasia stage 3 or more severe lesions (CIN3+) in Switzerland.

Methods

We conducted a cross-sectional study with women diagnosed with CIN3+ in Switzerland. Ten pathology institutes from six cantons and three language regions participated. We conducted HPV typing on formaldehyde fixed-paraffin embedded specimens from 2014 and 2015. Women enrolled in 2015 were asked to complete a questionnaire. We described frequencies of HPV types. We also compared demographic characteristics and socioeconomic status (according to the Swiss neighbourhood index of socioeconomic position, Swiss-SEP) in the CIN3+plus group with the Swiss National Cohort (SNC) in 2014 and compared risk factors for HPV infection with the Swiss Health Survey (SHS) in 2012.

Results

We included 768 biopsies from 767 women aged 17-81 years with CIN3+ in 2014 and 2015. Of these, 745 (97.0%) were positive for any HPV type, 5 (0.7%) were negative and 18 (2.3%) were not evaluable. Overall, 475/768 (61.8%) biopsies contained HPV 16 and/or 18 and 687 (89.5%) contained an oncogenic HPV type covered by the nonavalent HPV vaccine (16, 18, 31, 33, 45, 52, 58). In 2015, 273 women completed a questionnaire. Compared with the SNC, fewer women with CIN3+ were born in Switzerland (49.0 vs. 63.4%; p<0.001) and more were single (48.9 vs. 28.1%; p<0.001), but mean Swiss-Sep index was similar (64.6±10.8 vs. 64.6±10.8).
65.2±10.9; p=0.135). Amongst women with CIN3+, higher proportions reported ≥2 sexual partners in the last 12 months (15.4% vs. 4.1%), smoking (38.5% vs. 22.0%) and hormonal contraception use in the last 12 months (35.5% vs. 22.4%) than women in the SHS.

Conclusion

This is the first study of HPV in women with CIN3+ covering all three language regions in Switzerland. Women with CIN3+ have levels of socioeconomic position that are similar to the Swiss general population but higher levels of some risk factors for HPV. Surveillance of HPV types in CIN3+ lesions is feasible and can be used to measure the future impact of HPV vaccination on clinical outcomes.

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HPV VACCINE ACCEPTABILITY FOR DAUGHTERS AMONG AT-RISK WOMEN IN BRAZIL


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Background / Objectives

Human papillomavirus (HPV) vaccination recently became available in Brazil for school-aged girls through the Brazil National Immunization Program. We evaluated acceptability of HPV vaccination for daughters among women at high risk for cervical cancer.

Methods

Between January and November of 2016, we conducted a cross-sectional survey of 500 women with abnormal cervical cytology to examine predictors of HPV vaccination acceptability for daughters. Participants had no history of HPV vaccination, and were referred for first colposcopy at Barretos Cancer Hospital, a large tertiary care hospital in the state of São Paulo, Brazil.

Results

Most women had heard of prophylactic HPV vaccination (71%) and cervical cancer (95%). However, only around one third (39%) were aware of a vaccine to prevent cervical cancer. Most respondents (86%) indicated they would definitely get HPV vaccination for their adolescent daughters. Respondents were more likely to intend to vaccinate if a doctor recommended it (85% vs. 58% for nurse; p<0.001). Most (82%) indicated they would have their daughter vaccinated if it were available at school, and a notable proportion of respondents preferred to have their daughters vaccinated at a public health clinic (35%) or gynecologist’s office (41%). Correlates of intent to vaccinate included being married or in a domestic partnership (OR 2.41; 95% CI, 1.36-4.19), having the belief that HPV vaccination did not increase sexual activity (OR 2.36; 95% CI, 1.31-4.18), and that obtaining vaccination was not difficult (OR 1.86; 95% CI, 1.05-3.27).

Conclusion

HPV vaccination of adolescent daughters was highly acceptable to a group of at-risk Brazilian women, including through school-located programs. National vaccination strategies in Brazil should emphasize HPV vaccination as a free, accessible, and
effective tool to prevent cervical cancer and encourage physicians to discuss HPV vaccination with their patients.
STRATIFYING A SCREENING POPULATION INTO RISK CATEGORIES – PERSONALISING A CERVICAL CANCER SCREENING PROGRAMME

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Background / Objectives

Women screened for cervical cancer in Sweden and Norway are currently treated under a one-size-fits-all programme, which has successfully reduced the incidence of cervical cancer but relies on guidelines for scheduling screening. Utilising the information in the screening registries, guidelines could be replaced with individual risk estimates based on screening histories and ancillary information about the attendants. Adjusting the screening density at an individual level this way allows for increased observation of high-risk individuals while freeing up resources that would otherwise be spent on low-risk individuals that do not benefit from a high screening frequency. In this study, we applied a method for stratifying women into risk groups using their screening histories, based on the Swedish screening programme1, and validated these results with data from the Norwegian programme, adapting the method to make use of the detailed HPV tests and biopsy samples available there.

Methods

We previously developed a method for stratifying women into risk groups using their screening histories using the Swedish Quality Register for cervical cancer (NKCx). Each screening diagnosis was assigned a ‘risk score’ by an expert, and the history ‘risk score’ totals for each participant were computed by an algorithm that accounted for time and delay. The resulting method could identify some very high-risk individuals in the data (up to 15% of all Swedish cancer cases since 2001 were found in this group).

Results

Preliminary results show the same risk-identifying patterns in Norway as in Sweden, even though the screening programmes follow different clinical practices and guidelines. The ‘risk scores’ show exceptional probability of cancer in the upper ranges, and below normal risk in the lowest ranges. HPV status is often combined with low-risk diagnoses for prediction, but not high-risk, i.e. a result of normal is
treated differently if it’s HPV positive or negative, but the HPV status of a HSIL diagnosis doesn’t matter for predictive purposes.

Conclusion

Preliminary results show strong similarities between Norway and Sweden in terms of which screening patterns can be used to predict likelihood of cancer. This suggests that the methodology is robust, and that somewhat different screening guidelines do not affect the outcome of the algorithm as long as the data is accurate and complete. Furthermore, the addition of HPV status divides diagnoses into HPV groups, enabling more refined comparisons and predictions. As HPV tests become more widespread, the algorithm is likely to improve in prediction accuracy and detail.

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