Conclusions: HPV Allen infection is associated with Human Papillomavirus (HPV) infection, mainly by low risk types 6 and 11. Objectives: To characterize molecular variants of HPV among individuals diagnosed with RRP and to analyze the impact of the Long Control Region (LCR) heterogeneity upon viral early transcription. Methods: Cloning and sequencing of the entire LCR was performed for nucleotide variability analysis. Luciferase assays were conducted to analyze the viral transcriptional activity. Conclusions: We analyzed 14 biopsy specimens of RRP patients HPV-6 positive. Sequencing of the HPV-6 LCR revealed five genomic variants not described previously. Non-prototypic HPV-6vc related variants were more prevalent. Computational analysis showed that LCR nucleotide changes detected overlap potential binding sites for transcription factors such as FOXA-1, ELF-1 and GATA-1. Further, we observed that alterations detected strongly impacts transcriptional activity.

IS HUMAN PAPILLOMA VIRUS ASSOCIATED WITH SALIVARY GLAND NEOPLASMS? AN IN SITU HYBRIDIZATION STUDY

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Objective: HPV can infect cells of epithelial origin and is closely associated with carcinomas. Studies investigating its presence in salivary gland neoplasms are few and conflicting.

Methods: Detection of HPV types 16 & 18 was done using Digene HPV types 16 & 18 probe using in situ hybridization technique on a total number of 41 formalin-fixed, paraffin-embedded archival material (15 benign and 19 malignant salivary tumours, in addition to 7 control salivary tissue specimens). Benign salivary gland neoplasms consisted of 8 pleomorphic adenoma, one myoepitheloma and 6 Warthin’s tumour. Malignant salivary gland neoplasms included 7 adenoid cystic carcinoma, one epimyoepithelial carcinoma, one lymphoepithelial carcinoma, one lymphoma, 7 mucoepidermoid carcinoma, one myoepithelial carcinoma and one polymorphous low grade adenocarcinoma. Positive control slides (cervix carcinoma harbouring HPV) underwent the above mentioned procedure, to ascertain the validity of ISH kit and the accuracy of the technique. A violet colour was detected in superficial epithelial cells of cervix carcinoma.

Conclusions: An association exists between HPV infections and salivary gland neoplasms. However, given the sparse pattern of reactive cells, it cannot be confirmed that this virus is implicated in the aetiology of this group of tumours. Correlation was found between the incidence of HPV infection and histological differentiation of salivary gland neoplasms. Further studies with strong epidemiologic and clinical data and more number of samples would provide additional support for a causal association between HPV and salivary gland neoplasms. This might guide future cancer-prevention programmes involving vaccination to prevent HPV infection and screening for early detection of it.

DETECTION OF HUMAN PAPILLOMAVIRUS IN PLACENTA

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University del Valle. Faculty of Health, Science Basic School, Physiological Sciences Department. Cali – Colombia.

Objectives: To analyze human papillomavirus (HPV) DNA in placental samples obtained at delivery from 126 first-time pregnant women.

Methods: From December 2010 until November 2011, all the participants gave their informed consent to use the placental samples. The gestational age of the sampling was between the 36th and the 41th gestational week. All samples were treated upon arrival. DNA was extracted and DNA quality was verified by PCR on the β-globin gene with the PC03 and PC04 primers. HPV DNA was detected by molecular techniques according to the manufacturer’s protocol. The L1 of the viral genome region was amplified using 2 sensitive nested PCR strategies, both based on a general PGMY09/11 PCR, followed by a second nested PCR with either the GP5+/6+. Negative controls were included in every PCR reaction to rule out possible laboratory cross-contamination.

Conclusions: HPV DNA is detected in 13.5% of placenta samples. The presence of HPV DNA in placenta could be increases the risk of a neonate testing HPV-positive at birth.
Utility Loss with HPV-Induced Diseases in Men and Women

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Background: Variability with respect to utilities is a critical source of uncertainty in economic and clinical evaluations and the utility of each HPV-induced pathology needs to be elicited.

Objectives: This study was designed to measure the utility loss due to several HPV-induced diseases in both sexes in Italy. Risk factors influencing the viral transmission were also investigated.

Methods: In patients with ASC-US, CIN, cervical and anal-colorectal cancer, head and neck squamous cell carcinoma (HNSSC), and anogenital wart utilites were elicited using a standardised and computer-guided implementation of a time trade-off procedure. Utility were measured in six clinical research centres across Italy. A group of healthy subjects attending the same clinical centres was used as a control. The EQ-5D was administered to evaluate health conditions of respondents while another questionnaire was used to assess risk factor profiles.

Results: Overall 600 respondents were eligible for analyses: 465 patients (44.0 ± 16.3 years) and 135 controls (44.0 ± 13.2 years). The patients' perception of their health condition was equal to an EQ-5D score of 0.87 ± 0.22. The mean value of utilities corresponded to 0.83 ± 0.24, 0.78 ± 0.27, 0.83 ± 0.22, 0.81 ± 0.27, 0.58 ± 0.31, 0.51 ± 0.26 and 0.69 ± 0.30 for ASC-US, anogenital warts, CIN1, CIN2-3, cervical cancer, anal cancer and HNSSC, respectively. Having more than 5 sexual partners increased the risk of acquiring HPV-induced infections up to 2.52-fold, while for smoking or age at sexual debut ≤ 18 years the risk raised by about 1.62-fold. High levels of education were associated with a protective effect.

Conclusions: These data can be used as other HPV vaccination strategies, including the vaccination of pre-adolescent of both sexes in Italy.

Oral Administration of HPV-16 L2 Expressing Lactobacillus Casei Induces Systematic and Mucosal Cross-Neutralizing Effects in BALB/C Mice

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The human papillomavirus (HPV) minor capsid protein, L2, is a good candidate for prophylactic vaccine development because L2-specific antibodies have cross-neutralizing activity against diverse HPV types. Here, we developed a HPV mucosal vaccine candidate using the polyγ-γ-glutamic acid synthetase A (pgsA) protein to display a partial HPV-16 L2 protein (N-terminal 1-224 amino acid) on the surface of Lactobacillus casei (L. casei). The oral immunization with L. casei-L2 induced productions of L2-specific serum IgG and vaginal IgG and IgA in Balb/c mice. To examine cross-neutralizing activity, we used a sensitive high-throughput neutralization assay based on HPV-16, -18, -45, -58, and bovine papillomavirus 1 (BPV1) pseudovirions. Our results revealed that mice vaccinated with L. casei-L2 not only generated neutralizing antibodies against HPV-16, they also produced antibodies capable of cross-neutralizing the HPV-18, -45, and -58 pseudovirions. Consistent with previous reports, vaccination with HPV-16 L1 virus-like particles (VLPs) failed to show cross-neutralizing activity. Finally, we found that oral administration of L. casei-L2 induced significant neutralizing activities against genital infection by HPV-16, -18, -45, and -58 pseudovirions encoding a fluorescence reporter gene. These results collectively indicate that oral administration of L2 displayed on Lactobacillus casei induces systemic and mucosal cross-neutralizing effects in mice.

Distribution and Risk Association of HPV52 Variant Lineages Worldwide

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OBJECTIVES: Human papillomavirus (HPV) is a necessary cause of cervical cancer. Two (HPV16/18) of the 15 high-risk HPV types are covered by current vaccines. HPV52 is one of the non-vaccine-covered high-risk types, and is found in a higher proportion of cervical cancers in Asia compared to other regions of the world. This study aimed: (1) to establish a comprehensive set of HPV52 sequence data; (2) to conduct phylogenetic analyses for each lineage; (3) to identify genetic signatures for each lineage of HPV52 variants; (4) to identify mutations that confer an increased risk for the development of high-grade cervical lesion and cancer.

METHODS: HPV52-positive samples collected worldwide were transferred to a central laboratory for sequencing of the L1, LCR, E6 and E7 regions of HPV52. Maximum-likelihood trees were constructed for each gene region, and bootstrap resampled 1,000 times using the program MEGA5. Distribution and risk association were assessed by Chi-squared test or Fisher’s exact test with Bonferroni’s correction as appropriate.

CONCLUSIONS: Altogether, 611 specimens collected from 14 locations covering four continents were analysed. Four lineages (A-D) of HPV52 variants were observed. LCR region was better than L1 region for differentiating between sublineages B1 and B2. Genetic signatures were identified: lineage A retained CTT at nt 544-546 which were deleted in all other lineages, and had "G" at nt558 and "G" at nt 707. Lineage B had "T" at nt 217. Lineage C had “C-C-A-T” at nt 239-260-267-517. Lineage D was most diverged from others and had multiple substitutions including “T-C-T-G-T-C-G-G-G-C-T” at nt 14-95-227-270-417-425-763-779-816-874-885. HPV52 lineages showed predilection in geographical distribution. Lineage B was found significantly more common in Asia compared to other regions, whereas lineage A was more commonly found in non-Asian regions. Lineage A was associated with a lower risk. Nucleotide variations associated with higher risk were: lineage A G6218A and C6917A.

In conclusion, we found that HPV52 variants carrying different oncogenic risk exist and their prevalence rates vary across different geographical regions. These could be part of the reasons for higher disease burden attributed by HPV52 in Asia. The sequence variation and risk association should be considered when designing and applying HPV-based diagnostic assays and vaccines.
Objective: To determine the prevaccination prevalence and distribution of the five most common HR-HPV genotypes by age in Montenegrin women, in order to be able to predict and monitor the impact of HPV immunization in Montenegro.

Methods: Cervical cytobrush samples were collected from 1100 women, aged 14-82 years, attending the outpatient department at the Institute of Public Health of Montenegro, during the period from October 2008 till the end of February 2013. Cervical samples were analyzed for HPV E6/E7 mRNA by NucliSENS EasyQ® HPV assay (bioMérieux, France).

Results: The overall prevalence of HR-HPV 16, 18, 31, 33 and 45 was 15.0%, with the dominance of HPV genotype 16 (62%) over the genotype 18 (11%). The presence of HPV genotype 45 was found in 21%, HPV 31 in 4% and HPV genotype 33 in 2% of all HPV positive cases. Multiple genotype HPV infection was found in 15.29% of the HPV infected samples. The prevalence of the HPV multiple infection was highest at the age < 25 years and it was reduced at older ages. The most common combination was of HPV genotype 16 with HPV genotype 33 (23%). In general, prevalence of HR-HPV infection was highest in the age group 25 to 34 years (54.1%). It decreased progressively with increase of age, up to 4, 11% in the age group 45 to 54 years. After that age, there were no HPV positive cases. In our study, the youngest age group (<25 years) appears to had infection rates of 14.7%.

Conclusions: Out of all HPV positive samples, our results show that almost 3/4 of cases were HPV16/18 positive. Establishing this baseline distribution will help to monitor the early impact of HPV16/18 vaccination and to monitor possible changes in genotype-specific HPV distribution after vaccination has been introduced.

P 2-3
THE INCREASING INCIDENCE OF CERVICAL CANCER IN YOUNG WOMEN IN KOREA

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Objectives: This study was performed to examine trends in cervical cancer incidence in young women and to seek for solution to decrease cervical cancer in young women.

Methods: Cases of cervical cancer diagnosed between the year 2003 and 2010 in Korea were extracted from a database registered by National Cancer Information Center. Changes in incidence of cervical cancer were analysed by 10-year age group.

Results: Overall incidence, during 2003 to 2010, fell significantly from 9.0 to 7.7 per 100,000 person years. Peak incidence of cervical cancer was in those aged 40-49 years (27-29% of every year cases). New cases of cervical cancer with women aged 20-29 years were 104 of 4,315 (3.1%) in the year 2006, 114 of 3,616 cases (3.1%) in the year 2007, 141 of 3,888 cases (3.6%) in the year 2008, 129 of 3,733 cases (3.6%) in the year 2009, and 136 of 3,857 cases (3.5%) in the year 2010. Although the annual incidence of cervical cancer in women decreased between the year 2003 and 2010, cases of cervical cancer in young women aged 20-29 years increased by 31.4%(P=0.0211).

Conclusion: The screening for cervical cancer precursors by conventional cytology effectively reduces the overall incidence and mortality of cervical cancer. But the prevalence of cervical cancer in young women is increasing. It is necessary for young women to receive cervical cancer screening, HPV vaccination and other public health policies, for example sexual health education.

P 2-4
HPV-INDUCED CERVICAL LESIONS IN YOUNG BRAZILIAN POPULATION - ANALYSIS OF CERVICAL BIOPSIES BETWEEN 2007 AND 2011

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Objective: The aim was to know the prevalence of cervical cancer and its precursor lesions in the Brazilian population between 11 and 29 years old.

Methods: This is a retrospective study of the histopathological reports of 126,945 cervical biopsies performed between 2007 and 2011, taken from the DATASUS/ SISCOLO database. The distribution of the pathologic alterations was compared throughout the study period, in the three age groups (adolescents, young adults and adults).

Results: The CIN I (Cervical Intraepithelial Neoplasm grade I) was the most frequent lesion observed, showing increase rates in all age groups. The greatest increase in CIN I occurred among adolescents (86.0/1000 tests), followed by the young adult group (57.1/1000 tests) and the adults (36.6/1000 tests). During the same period the frequency of more severe lesions showed an ascending trend directly proportional to the age of the woman. The raise in the incidence rate of CIN II was 23.7/1000 in adults, 17.4/1000 in young adults and 11.7/1000 in adolescents, while the increase of the CIN III was 18.6/1000, 6.6/1000 and 7.6/1000, respectively. Analyzing the trend in the cervical cancer rates, it was observed that the age group between 20 and 24 years old had the highest increase from 2007 to 2011 (53%). In the adolescent group 23 cases of epidermoid carcinoma and 11 cases of adenocarcinoma were identified, in the period.

Conclusions: The trend in the number of positive cases of CIN I, CIN II, CIN III and malignant neoplasms showed an increase between 2007 and 2011. The early detection of precursor lesions is an important tool for the prevention of the cervical cancer.
PREVALENCE AND DISTRIBUTION OF HUMAN PAPILLOMAVIRUS INFECTION IN ARAB WOMEN IN STATE OF QATAR

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OBJECTIVES Human papillomavirus (HPV) are the most commonly known sexually transmitted agents. To date, few reports are available on distribution of most prevalent and variants types of HPV in Arab women. Therefore, the aim of this study was to determine the distribution of HPV types among Arab women being subjected to routine Pap smear test in State of Qatar.

METHOD 2600 pap smears have been collected in ThinPrep vials (BD SurePath™) from the Arab women seeking routine gynecological care visiting Women’s Hospital, Hamad Medical Corporation (HMC), Doha, Qatar. All the samples were transported to the laboratory in icebox container. Viral DNA from ThinPrep samples was extracted by QIAamp MinElute virus spin kit according to manufacturer’s instructions and was screened for HPV DNA by real-time PCR using L1 HPV specific (GP5+/6+) primers. The type specific distribution of the viruses was determined by HPV high and low risk typing RT-PCR kits (Sacace Biotechnology, Italy) and PCR-based sequencing. Real-time PCR amplification was carried out in ABI 7500 real-time PCR (Applied Biosystems).

RESULTS Based on the collected data, HPV DNA was detected in 125 (4.8%) Arab women, where HPV DNA was highest (42.4%) in 30-49 age groups. Among HPV DNA positive Arab women, 37 (4.8%) were Qatari and 39 (4.9%) non-Qatari. Genotyping was done in all HPV positive samples by RT-PCR kits and DNA based sequencing and 17 different HPV genotypes were detected, comprising high-risk and low-risk genotypes.

IMPLICATIONS AND IMPACT The study shows that the prevalence of HPV infection in Arab women’s in Qatar is among the highest in the Arab world compared with previous reports. However, more extensive population-based studies should be undertaken before implementing HPV vaccination.
FREQUENCY OF HPV HIGH AND LOW RISK IN BRAZILIAN SAMPLES

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Objective: According to the World Health Organization, the main risk factor for cervical cancer is HPV infection. The incidence and mortality rates for cervical cancer in Latin America are among the highest in the world. In Brazil, HPV 16 is the most prevalent in all regions followed by type 18, the second most prevalent in the North, Southeast and South regions. The hybrid capture test has been the most important HPV diagnostic assay for the last decade and is still the most frequently used diagnostic test HPV worldwide, although there are other diagnostic methods currently available in the market and based on PCR reaction. The aim of this study is to determine the frequency of HPV high and low risk in Brazilian samples using hybrid capture method.

Method: From April 1 to April 30, 2013, 4324 genital samples (3556 from female and 768 from male patients) were collected using Digene STM (Qiagen, Hilden, Germany). The average age of female patients was 32 years and 33 years for male patients. Hybrid capture is a hybridization assay designed for aggregate detection of 13 HPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68) using a mixture of unlabelled single-stranded full-genome-length DNA probes. The detection of high and low risk HPV by hybrid capture method was performed at Molecular Diagnostics laboratory (DASA) using the hc2 HPV DNA test kit (Qiagen, Hilden, Germany) and Rapid Capture System as automated platform. Samples with a relative light unit per cutoff (RLU/CO) ratio higher than 3.00 were considered positive, and samples with an RLU/CO value of less than 1.00 were considered negative. Samples with RLU/CO between 1.00 and 3.00 were considered as low positive.

Conclusions: Table 1 describes the results found in all samples tested. The overall frequency of HPV high and low risk found with RLU/CO higher than 3.00 and using hybrid capture as detection method was: 29% in female samples and 19% in male samples. We found that the HPV high risk were more frequent in female samples and HPV low risk were more frequent in male samples. Low positive results were found in female (11%) and male (9%) samples and should be further investigated by other molecular techniques.

Table 1. Frequency of HPV high and low risk in female and male samples.

<table>
<thead>
<tr>
<th>Result</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative to high and low risk</td>
<td>≤1.00</td>
<td>56%</td>
</tr>
<tr>
<td>Low positive to high and low risk</td>
<td>1.00 to 3.00</td>
<td>11%</td>
</tr>
<tr>
<td>Positive only for low risk</td>
<td>≥3.00</td>
<td>7%</td>
</tr>
<tr>
<td>Positive only for high risk</td>
<td>≥3.00</td>
<td>22%</td>
</tr>
<tr>
<td>Positive to high and low risk</td>
<td>≥3.00</td>
<td>5%</td>
</tr>
</tbody>
</table>

HPV STATUS IN MENOPAUSAL WOMEN. IS CERVICAL SCREENING BY CYTOLOGY ENOUGH?

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Introduction: Menopausal women pose difficulties in cervical screening and colposcopic assessment due to the atrophy of the cervix and vagina. Although traditionally HPV related conditions are thought to affect younger women, a proportion of menopausal women will continue to harbour the virus, while some may first contract it later in life through a new sexual relation.

Methods: We searched our archive for menopausal women undergoing cervical screening cytology and HPV typing. We collected data on their age, HPV type and cytology result.

Results: We identified 313 women of menopausal status in our database, aged 49 to 79 years. 73 (23%) women were positive for HPV, of which 51 for hrHPV and 22 for lrHPV. As far as cytology is concerned, 227 had a negative result, 20 had ASCUS, 59 had a LGSIL and 6 had a HGSIL. 12.3% of those with a normal result were positive for HPV as were 25% of those with ASCUS. Of those with LGSIL and HGSIL, 59 and 83% were HPV positive respectively.

Among those with LGSIL, the commonest HPV types identified were HPV 16, 51 and 42, at a rate of 19% each. Among those with ASCUS five patients harboured a high risk HPV type (16, 52, 70) and of those with normal cytology, 27 had a hr HPV type.

Conclusions: HPV typing could help identify those older women that should undergo a through colposcopic and histological assessment in the presence of a low grade or ASCUS cytology result, while at the same time providing reassurance if a low risk type is identified. Furthermore, given the high percentage of false negative cytology results, HPV typing could provide additional screening information and help triage those women with a risk of progression to high grade cervical disease and cancer.

HIV PREVALENCE IN WOMEN WITH HPV 16 AND 18 INFECTION IN GALICIA, SPAIN.

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OBJECTIVE: A higher prevalence, incidence and persistence of HPV infection has been strongly associated with HIV seropositivity by some authors. HIV seropositivity has also been linked to multiple-genotype infection and to high-grade and low-grade cervical intraepithelial neoplasia, and invasive cervical carcinoma. Nevertheless, evidence on the role of HPV in predisposition to the acquisition of HIV infection and dissemination through its disruption of the epithelium integrity and the mucosal immune system is still limited. Knowledge of baseline HIV infection in HPV infected women could contribute to evaluate the HPV vaccination impact in the spread of HIV. The objective was to know the HIV prevalence in women with genital HPV 16/18 infection with and without cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in Galicia, Spain.

METHODS: We conducted a prospective study including consecutive detections of HPV 16/18 during 2009-2010 in women attending Gynecological Units of two University Hospitals of Galicia (Spain) for cervical cancer screening. We also enrolled women with CIN2+ related to HPV 16/18 during 2011-2012. The study was approved by ethics committee of clinical investigation of Galicia (Santiago de Compostela, Spain). Four hundred and eighteen women agreed to be enrolled but only 292 (69.9%) wanted to be tested for HIV infection.

CONCLUSIONS: Prevalence of HIV was 1/148 (0.67%) in women without CIN2+ and 9/144 (6.25%) in women with CIN2+. It is important that patients understand the importance of knowing if they are coinfected with HPV 16/18 and HIV, specially in case of CIN2+ detection.
THE RELATIONSHIP BETWEEN PREVALENCE AND GENOTYPING OF HUMAN PAPILLOMAVIRUS 16/18 WITH CERVICAL ABNORMALITIES BY AGE GROUPS

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Objectives: To investigate the prevalence of HPV 16 and / or HPV 18 in different age groups, and the association between such parameters with the severity of cytological and histological cervical diagnosis.

Methods: This is a cross-sectional study including 331 women with cytological diagnosis at their referral smear. Of them, 238 women had histological diagnostics. HPV types detected in different cytological and histologic abnormalities were compared in different age groups: 1<30, 2 (30-39), 3 (40-49), and 4 (> 50). HPV DNA was detected and genotyped by PCR reverse line blot hybridization assay.

Results and Conclusions: Among women with cytological diagnosis (331), 48 were normal (14.5%), ASC-US was detected in 69 (20.8%), ASC-H in 23 (7%), LSIL in 43 (13%), AGC-SOE in one case (0.3%), HSIL in 141 (42.6%) and invasive carcinoma in six cases (1.8%). Of 238 women with histological diagnosis; 51 (21.4%) were diagnosed with cervicitis, 80 (33.6%) with CIN 1, 48 (20.2%) with CIN 2, 50 (21%) with CIN 3 and 9 (3.8%) with invasive carcinoma. HPV prevalence was 82.5% (273/331) in women with abnormal cytology. When only women with histological diagnostic were considered, HPV prevalence was 84.9% (202/238). Concerning HPV types, HPV 16 and/or 18 were detected in 48.7% and 48% of women with cytological and histological diagnostics, respectively. The analysis between specific HPV 16 and / or 18 with cytological and histological diagnoses by age groups showed that women younger than age 30 presented with a higher risk of having more severe cytological (OR: 2.12 CI 0.98 to 4.59) and histological abnormalities (OR: 3.21 CI 1.21 to 8.59) than women in the same age group infected by different HPV types. The risk to develop CIN 2 or a worse lesion seems to be age and HPV genotype related. These data suggest that cervical neoplasia related to HPV 16 and HPV 18 may behave differently in the process of carcinogenesis.

GENITAL HPV INFECTION AND PROGRESSION TO EXTERNAL GENITAL LESIONS: THE HIM STUDY

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Objectives: To assess genital HPV infection progression to corresponding type-specific external genital lesions (EGLs).

Methods: A prospective analysis was conducted among 2,754 men enrolled in the HPV Infection in Men (HIM) Study, who had two or more follow-up visits scheduled 6-months apart from 2009-2012. At each visit, visual inspection of the external genital skin was conducted, and exfoliated cells were collected from the normal genital skin using swabs, which then were used to identify 37 mucosal HPV types using PCR and Linear Array genotyping protocol. Visually distinct EGLs were biopsied and subjected to pathological evaluation. We assessed the time from the first HPV-positive visit to either EGL incidence or last visit. EGL was categorized by pathological diagnoses: condyloma, suggestive condyloma, penile intraepithelial neoplasia (PeIN), or other/non-HPV lesion. HPV type-specific incidence of EGL was determined from prevalent or incident HPV infection estimating incidence rates and 12-month cumulative incidence. The proportion of HPV infections that progress to development of type-specific EGL was also calculated along with median time to EHL development.

Conclusions: There were 198 men with an incident EGL during follow-up, 10 were diagnosed with PeIN, 88 with condyloma, 85 with suggestive of condyloma, and 68 with other/non-HPV infections. Among men with incident or prevalent HPV infections, the highest rate of progression to a pathologically confirmed EGL was for HPV 11 (30% progressed) and 6 (26.6% progressed). Fewer infections with HPV 16 (4.4%) and 18 (2.1%) progressed to a pathologically confirmed EGL. Median time to lesion development was shortest for HPV 11 (1.3 months), HPV 18 (3.1 months), and HPV 6 (6.3 months). A total of 12.6% of vaccine-preventable HPV infections (types 6, 11, 16, or 18) progressed to a pathologically confirmed EGL. Median time to lesion development was shortest for HPV 11 (1.3 months), HPV 18 (3.1 months), and HPV 6 (6.3 months). The proportion of HPV infections that progresses to the development of type-specific EGL was also calculated along with median time to EHL development.

PREVALENCE OF HUMAN PAPILLOMAVIRUS AND HRHPV E6/E7 mRNA EXPRESSION IN 52 GREEK PATIENTS WITH SCC OF THE ORAL TONGUE

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Objective: Several investigators have reported detection of HPV infection in squamous cell carcinoma of the oral cavity (OSCC). HPV integration into the host cell genome however has been reported as a causal factor in head and neck carcinogenesis. The objective of this study was to indentify HPV genotypes and E6/E7 mRNA expression from hrHPV types in Greek patients with SCC of the oral tongue.

Methods: 52 biopsies collected in Thin Prep medium from patients with SCC of the oral tongue were analysed using the PapilloCheck HPV for the type-specific identification of 24 types of HPV and NuClSENS EasyQ HPV 1.1 was performed for the qualitative detection of HPV E6/E7 mRNA of five high risk HPV types (16, 18, 31, 33 and 45).

Conclusions: The age range was 19-75 years old (y.o) with a mean age of 51 y.o. 15 patients were female and 37 were male. The overall HPV prevalence was 11.5% (6/52), 7.7% (4/52) were infected with hrHPV type and 3.8% (2/52) with lrHPV type. E6/E7 mRNA expression from hrHPV types was detected in 9.6% (5/52) of the samples. HPV6 was found in two cases in one of which E6/E7 mRNA expression from HPV16 was detected. HPV16 was found in three cases. E6/E7 mRNA expression from HPV 16 was detected in all three cases. HPV18 was detected in one case along with E6/E7 mRNA expression from HPV 18. The results of this study show that oral tongue SCCs are associated with HPV infection and E6/E7 hrHPV mRNA expression, confirming the international data.
HPV INFECTION IN HIV POSITIVE WOMEN VACCINATED WITH A QUADRIVALENT HPV VACCINE

Moses E, 1,2 Blitz S, 3 Coutlee F, 4 Walmsley S, 1 Loutfy M, 6 Smail F, 4 Trottier S, 7 Wobeser W, 4 Ogilvie G, 8 Dobson S, 6 Money D, 8

1 University of Toronto, 2 Women’s Health Research Institute, 3 University Health Network, 4 University of Montreal, 5 Women’s College Research Institute, 6 McMaster University, 7 Laval University, 8 University of British Columbia

Objective: To determine rates of new HPV infection in women vaccinated with the quadrivalent HPV vaccine.

Methods: Data was collected as part of an open label multi-centered, longitudinal study of the immunogenicity and safety of a quadrivalent HPV vaccine in HIV positive women. For this sub-analysis, the presence of HPV DNA in cervical specimens was assessed at baseline and subsequent visits at 6, 12, 18 and 24 months. Genotype specific HPV infection was determined by Linear Array. Incident infection was defined as undetectable HPV antibody levels and the absence of HPV DNA at baseline followed by the presence of at least one HPV type covered in the quadrivalent vaccine (6,11,16,18) at any of the follow up visits, while persistent infection was presence of the same HPV DNA type at least 2 consecutive follow up visits. HPV antibody (AB) levels for 6, 11, 16, 18 were determined by Merck cLIA assay.

Conclusion: 136 of 351 sexually active women enrolled to date were included in this secondary analysis. Median age was 40; 52% were black, 36% white, 7% aboriginal. Median time since HIV diagnosis was 8 years and 10% were co-infected with Hepatitis C. 95% of participants were on HAART medication at baseline, 70% had undetectable viral load, median CD4 was 500 (IQR: 390-684). 7 incident and 2 persistent infections were observed in this population of women. CD4 Nadir was lower in subjects with HPV infection compared to those without infection: 69 (IQR 31-184) vs 224 (IQR 120-310). A higher number of subjects with HPV infection also reported having at least one new sexual partner since baseline compared to subjects without infection; 25% vs 5% respectively.

Rates per HPV Type

<table>
<thead>
<tr>
<th>HPV Type</th>
<th>Incident Events</th>
<th>Persistent Cases</th>
<th>Person Years of Follow-up (PYF)</th>
<th>Incidence Rate 95% CL</th>
<th>Persistent Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>65</td>
<td>2</td>
<td>124.5</td>
<td>1.61 (0.9-2.1)</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>114</td>
<td>1</td>
<td>173.0</td>
<td>0.58 (0.05-1.3)</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>82</td>
<td>1</td>
<td>117.1</td>
<td>0.85 (0.02-2.4)</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>117</td>
<td>5</td>
<td>162.9</td>
<td>3.07 (1.00-7.16)</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Compared to previously published studies there was a higher rates of incident infections were observed in this population of HIV positive women. Future analysis of the full cohort will allow us to determine whether these high persistent rates of HPV18 infection reflect a suboptimal vaccine protection in the immune compromised populations.
ANAL HPV PREVALENCE AND ANAL CYTOLOGY AMONG HIV-INFECTED AND HIV-UNINFECTED MEN WHO HAVE SEX WITH MEN

DOINA MG1, BENEVOLO M2, VOCATURO A1, GIGLIO A1, MORETTO D1, LATINI A1, ROLLO F1, RONCHETTI L2, PIMPINELLI F1, CRISTAUDO A1, GIULIANI M1

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Objectives: HPV is responsible for 85% of anal cancers. Recently, anal cancer incidence has been increasing in developed countries, particularly in men who have sex with men (MSM). Cytology may be a useful tool for the detection of anal lesions.

Methods: MSM attending an STI clinic in Rome (Italy) were enrolled. Anal samples were collected with a Draco swab and stored in PreservCyt (Hologic). The Linear Array Genotyping Test (Roche Diagnostics) was used for HPV genotyping. Liquid-based cytology was classified following the Bethesda 2001 guidelines.

Conclusions: 438 HIV-uninfected (median age 32 yrs, IQR: 27-39) and 121 HIV-infected MSM (median age 40 yrs, IQR 33-46), without anogenital warts, were enrolled. Most of the individuals were Caucasian (97.0% and 89.2%, respectively). HPV prevalence, overall (92.6% vs. 72.4%, p<.001) and by High-risk (HR) HPV types (79.3% vs. 56.0%, p<.001), was significantly higher among HIV-infected than uninfected MSM. HPV-multiple infections were evidenced in 48.2% of the HIV-negative MSM and 75.2% of the HIV-positive counterpart (p<.001). The most common HPV overall and among HR types was HPV16 in both populations (17.6% among HIV-negative; 24.0% among HIV-positive MSM). The second most prevalent HR-HPV was HPV53 (10.3%) for the HIV-uninfected and HPV59 (18.2%) for the HIV-infected individuals. HPV6 and 84 were the most frequent Low-risk types in both cohorts. Anal cytological abnormalities were found in a significantly higher proportion of HIV-infected MSM (46.5% vs. 27.9%, p<.001). HSILs were exclusively observed among the HIV-infected individuals (2.6%).

Conclusions: A high prevalence of anal HPV infection and cytological abnormalities was evidenced in both populations, although HIV-infected MSM showed a significantly higher rate of infection and abnormal cytology. HPV type-specific profiles of the two groups were different, especially for HR-HPVs.

USING A SYSTEM DYNAMICS SIMULATION MODEL TO DETERMINE HUMAN PAPILLOMAVIRUS VACCINATION EFFECTIVENESS


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Objectives: To integrate the natural history of human papillomavirus (HPV) infections with HPV vaccine trial data and to demonstrate population effects at varying vaccination strategies.

Methods: We used system dynamics modeling to simulate HPV exposure and incidence of Cervical Intraepithelial Neoplasia 2 and higher (CIN2+) following HPV vaccination in a ‘catch-up’ population of women (18-26 years old). Using our model, we simulated a cohort of 100,000 HIV naïve thirteen-year-old women who were followed for the development of CIN2+ over 50 years, through three phases: infection, classification, and surveillance. We then varied the parameters that are most important to vaccine policy analysis: the upper age limit for vaccination and the targeted percent vaccinated, and estimated the cumulative incidence of CIN2+ lesions under these different vaccination strategies.

Conclusions: Our model shows that the incidence of HPV 16/18 infections and CIN2+ lesions are lower at higher vaccination rates. When 50% of the catch-up population is vaccinated, we observe 24,449 HPV 16/18 infections and 7,924 HPV 16/18-associated CIN2+ lesions after 50 years of follow-up. Increasing the percent of the catch-up population vaccinated from 50% to 100% prevents an additional 13,962 HPV 16/18 infections (or 57% reduction) and 2,241 CIN2+ lesions (28% reduction). In order to prevent an additional CIN2+ lesion in a population of 100,000 women, when 50% of the catch-up population is vaccinated, the number-needed to vaccinate (NNV) is 14. This increases to 21 assuming 100% coverage. Our model also depicts a decreased benefit of extending the upper age limit for vaccination of the catch-up population. The decreased benefit of vaccinating the catch-up population, compared to the benefit of vaccinating women younger, argues for strategies that preferentially emphasize vaccination of younger women. Our model demonstrates a novel approach to integrating the natural history of HPV infections with clinical trial data in order to demonstrate population effects at different vaccination strategies, which in reality, could never be replicated due to prohibitive time and cost.

HPV POSITIVITY IN TRIPLE-NEGATIVE BREAST CANCERS

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Objectives: Breast cancer is the main tumor among females; the viral etiology has been recently evaluated alongside with individual factors considered for the screening. HPV infection represents an interesting option because of the currently available preventive tools. The evidence is controversial owing to several factors, including variable prevalence of the infection, poor evaluation of the histological and the receptor pattern. We carried out a retrospective study to assess the relationship between HPV and mammary neoplasia in a high prevalence geographical area.

Methods: 40 paraffin-embedded biopsies of triple-negative (estrogen receptor/progesterone receptor/HER2–negative; TNBC) breast tumors were compared with 40 breast cancers characterized by a different receptor positivity. DNA was amplified using the PCR INNO-Lipa-Genotyping-Extra.

Conclusions: The majority of the neoplasias were ductal (85.0%), followed by lobular (6.3%), and others (8.7%). TNBC were the most undifferentiated, with a proportional 1, 2 and 3 grading of 0.0%, 27.5%, and 72.5% vs. 15.0%, 70.0%, and 15.0% of the controls (P<0.05); no differences were found for the mean ± SD age (58.8±18.2 vs. 59.2 ±15.6, for TNBC cancers vs. controls, respectively). HPV prevalence was 7.5%. HPV positivity was detected only for TNBC tumors (15.0%;P = 0.026). The most frequent HPV genotypes were 16 (28.6%), 31 (14.3%), 45 (14.3%), 52 (14.3%), 66 (14.3%); the only co-infection detected was HPV 6 with HPV 66. The prevalence found is similar to the median prevalence of the 50 cross-sectional studies carried out globally. Our study is the first one focused on the relationship between HPV infection and TNBC, showing its potential etiopatho-genetic role in this kind of tumors.
PREVALENCE OF HPV AND CHLAMYDIA TRACHOMATIS INFECTIONS IN UNDOCUMENTED IMMIGRANT WOMEN IN MILAN, ITALY

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3 NAGA Center, Milan, Italy

OBJECTIVES: This study aimed at evaluating the molecular epidemiology of Human Papillomavirus (HPV) and Chlamydia trachomatis (Ct) infections among undocumented immigrant women in Milan (North Italy) who are not involved in the cervical cytolgy screening program or routine gynaecological visit in the formal healthcare system. Molecular assays for the identification of HPV-DNA and Ct-DNA were performed in urine samples.

METHODS: 390 urine samples were collected from immigrant women (median age 38 yrs; range 18-65 yrs) attended the NAGA Center in Milan between June 2012 and May 2013. Informed consent was obtained by all participants. HPV and Ct infections were detected using PCR assays amplifying an ORF L1 segment and a cryptic plasmid segment respectively. All HPV-DNA positive samples were subjected to viral typing using a RFLP assay.

All HPV positive women were called to perform a Pap-test, evaluated using Bethesda System Classification.

CONCLUSIONS: About 560 immigrant women visited the NAGA Center during the study period and 390 participated in the study. The acceptability rate was 70%. The enrolled women belonged to 36 different nationalities and 21% were immigrants from less than one year. Most of the women came from South-America (34.1%) and Eastern Europe (31.3%). Over 70% of women were married or had a stable partner, were not using contraception and had at least one pregnancy. The prevalence of HPV infection was 22.6% and more than 50% of the infections were sustained by HR-clade genotypes.

The prevalence of Ct infection was 7.2%, higher in young women (≤25 years) than in those aged >25 years (14.3% vs 6%, p<0.05). 2% of women were HPV/Ct co-infected. 38.6% of HPV positive women have undergone Pap-test: 82.3% had normal cytology; only one woman (2.9%) was diagnosed with H-SIL. These preliminary data indicate that the ongoing study is highly accepted from undocumented immigrant women living in Milan. The prevalence of HPV and Ct infections is lower than that observed in immigrant women analysed in other countries. A based-urine test seems to increase screening uptake among women at increased risk for cervical cancer or reproductive tract infections compared with Pap-test, sometimes avoided because of the dislike of physical examination or religious reasons.

MIGRAINE FOLLOWING HUMAN PAPILLOMAVIRUS (HPV) VACCINATION

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2 Department of Medical Informatics, Erasmus Medical Centre Rotterdam, The Netherlands

Objectives: Since the introduction of HPV vaccination in the Netherlands (Cervarix®), a notable event reported in the passive surveillance system is migraine. The reporting rates of migraine in 2009 and 2010 were 0.09 and 0.12 per 10,000 administered doses, respectively. Research of a possible association between HPV vaccination and headache is necessary to maintain trust in the NIP. We conducted this analysis in addition to earlier research where incidences in pre- and post-vaccination years were compared.

Methods: Potential incident migraine cases were selected from a longitudinal observational electronic database of medical records from Dutch GPs (Integrated Primary Care Information (IPCI), Erasmus MC Rotterdam). Potential cases were selected if the record contained the ICD-code N89 or ‘migrai*’ in the free text within 2009-2010. Selected cases were manually validated and coded. Girls born in 1993-1997 (i.e. who were eligible for HPV vaccination in 2009-2010) from the IPCI database were linked to the vaccination registry (Praeventis, RIVM) to determine their HPV vaccination status. Self-controlled case series (SCCS) analysis was used to examine a possible association between HPV vaccination and newly diagnosed migraine.

The high-risk period was defined as 6 weeks after each dose. Furthermore, a cohort analysis was conducted to compare monthly incidences of headache in vaccinated girls with the incidence in unvaccinated girls.

Conclusions: No statistically significant higher risk of migraine was found in high-risk periods versus non-high-risk periods, with a RR for certain migraine of 4.3 (95% CI 0.68-26.6) and for certain probable migraine of 2.9 (95% CI 0.71-11.7). Furthermore, IRRs for migraine in monthly periods following vaccination compared to migraine in unvaccinated girls ranged from 0.0 to 3.0, however none was statistically significant. Thus, no statistically significant association between HPV vaccination and migraine was found using different kinds of analysis. However, numbers of cases were rather low. Including higher number of cases will be possible in the future to further exclude a possible association. Overall, to validate signals from passive surveillance systems, linkage studies using electronic databases are an important tool.

THE ITALIAN “CALL CENTRE PROJECT” FOR HPV-VACCINATION: PRELIMINARY RESULTS FROM THE PILOT EXPERIENCE IN CALABRIA REGION AND GENOA

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1 ASL3 Genova-Italy; 2 ASP Reggio Calabria-Italy; 3 ASP Vibo Valentia-Italy; 4 Dip. Tutela della Salute, Regione Calabria-Catanza; 5 Direzione Medica, GSK Spa, Verona-Italy

Four years after the start of the Italian HPV-vaccination programme, national coverage rate (CR) for 3 vaccine doses is 68.5%, 67.8% and 66.3% for the 1997, 1998 & 1999 cohorts, resp. This is lower than expected and lower than the objectives of the National Immunisation Plan 2012-2014. Objectives: The “Call Centre Project” (CCP) has been implemented to collect reasons for non-adherence to HPV vaccination, provide information on HPV-vaccination to mothers of eligible, but non-responders, girls and improve the CR in these target cohort adolescents.

Methods: A Protocol Agreement outlining the project’s rationale, objectives, actions, responsibilities and duration was finalised between Calabria Region (CaR)/Local Health Unit 3 Genovese (LUH3) and GlaxoSmithKline (GSK). GSK contracted the external provider Merquiro (Mq) for calling the non-responders’ girls’ families/mothers. The CaR/LUH3 defined the training programme of Mq’s operators and the call flow, identified the non-responders’ girls (1997/1998 cohorts in Calabria and 1997/2000 cohorts in Genoa), and organised the vaccination/counselling sessions at the vaccination centres.

The CCP started in March 2012 in CaR and July 2012 is in progress. We present preliminary results to date (June 2013).

Results: In Calabria Mq made 9000 phone calls in 6 months, followed by appointments for vaccination and counselling sessions in 24.5% and 16% of cases, resp.; the CR’s increase for the first HPV vaccine dose was 6.4% (total CR=74.8%) and 7.4% (total CR=72.8%) for the 1997 and 1998 cohorts resp. In Genoa Mq contacted 991 families in 6 months, followed by booking of vaccination and counselling session in 23.1% and 18% of cases, resp.; the CR’s increase for the first HPV vaccine dose was 2.5% (total CR=76.8%) and 5.9% (total CR=68.9%) for the 1997 and 2000 cohorts, resp. The reasons for non-adherence cited by the girls’ mothers are: vaccine too new, contrary to vaccines, doctor not in favour, girl too young, uncertain vaccine duration and safety.

Conclusion: These preliminary results show that a such type of service can improve the HPV-vaccination adherence of non-responders girls.
UNIVERSAL VACCINATION AGAINST 6/11/16/18 HPV-RELATED DISEASES IN SWEDEN: TOWARDS HPV DISEASE CONTROL?

Bresso Xć, Cassel Tć and Adam Mć.
1: Sanofi Pasteur MSD, Lyon, France - 2: Sanofi Pasteur MSD, Solna, Sweden.

OBJECTIVES: Human Papillomavirus (HPV) diseases are universal while HPV vaccination. The International Agency for Research on Cancer acknowledges that HPV is a human carcinogen affecting both sexes. As in Australia and Canada, in Sweden, vaccination coverage of girls is high and adding boys to the vaccination programme would offer the chance to adequately control circulation of 6/11/16/18 HPV virus, in addition to directly protecting boys. This study aimed to estimate the impact on 6/11/16/18 HPV prevalence of vaccinating boys and girls in Sweden with the quadrivalent HPV vaccine.

METHODS: A dynamic transmission model previously described and peer-reviewed was adapted to the Swedish setting. 85% vaccination coverage of girls aged 11-12 in addition to a catch-up up to age 18 (80% cumulative coverage assumed) was compared to a universal vaccination strategy (same age bands and vaccination coverage for boys and girls; i.e. 85% cumulative coverage for age 11-12 and 80 for catch-up). 90% compliance was assumed. Sensitivity analyses were performed to handle uncertainty related to vaccine’s duration of protection, calibration and coverage rates for boys. Results are presented vs. a screening only situation and vs. a girls’ only vaccination programme; over the next decades.

RESULTS: By adding boys to the vaccination program, 61% and 51% additional decrease of HPV16/18 prevalence was predicted in a steady-state situation (among boys and girls respectively) compared to vaccination of girls only. This could lead to a 93% and a 95% reduction of HPV 16/18 prevalence in boys and girls respectively versus the current situation. Similarly, 54% and 37% additional decrease in HPV6/11-related warts incidence was predicted in a steady-state situation (among boys and girls respectively). This could lead to quasi-eradication of HPV16/11-related warts, as seen in real life setting.

Results were mostly sensitive to vaccine duration of protection.

CONCLUSIONS: Maximizing coverage rate of HPV vaccine through gender neutral (universal) policy would allow adequately controlling HPV 6/11/16/18 virus circulation and participating to the overall goal of disease elimination.

Results are in line with conclusion from other models performed in similar setting that concluded to a quasi-elimination of strains included in vaccines.

ROMANIAN HPV VACCINATION STATUS: JUN 2010 - JUN 2013

Streinu-Cercel Oć,1,2 Streinu-Cercel Ać,1,2 Rafila Ać,1 Pana Ać,1 Pistol Ać,4 Sandulescu Mć,1 Streinu-Cercel Ać1,2
1 Carol Davila University of Medicine and Pharmacy, Bucharest, Romania - 2 National Institute for Infectious Diseases *Prof. Dr. Mătei Bal?, Bucharest, Romania - 3 Romanian Ministry of Health, Bucharest, Romania - 4 National Institute of Public Health, Bucharest, Romania

Objectives. We performed a study to determine the human papillomavirus (HPV) vaccine status in Romania following the introduction of HPV vaccination in the national immunization schedule.

Methods. We prospectively collected all data regarding the national immunization schedule between June 2010 and June 2013.

Conclusions. In June 2010 a large number of girls (62,759) had already been vaccinated through the national immunization schedule: 32,344 (52%) ages 12-14, 16,100 (26%) ages 15-19, 10,154 (16%) ages 20-24 and 4,161 (7%) ages 24 and over. By the end of 2010, the cumulative number of vaccinated girls increased rapidly, to 97,514. Over the following years, the numbers increased at a slower pace, with 119,532 girls vaccinated by December 2011, 124,298 by December 2012 and 125,533 (a 2-fold increase) by June 2013 – 38,179 (30%) ages 12-14, 31,515 (25%) ages 15-19, 30,750 (24%) ages 20-24 and 25,089 (20%) ages 24 and over.

Based on the results of the 2011 national census, it becomes apparent that in June 2013, 14% of the girls with ages 12-14 years had been vaccinated, compared with 6% of ages 15-19, 5% of ages 20-24, and less than 1% of women ages 24 and over.

At this point, we consider it useful to reevaluate the objectives of the national immunization schedule and devise a novel approach for attaining the critical mass effect, highly important in the strategy for reducing the transmission of HPV.

PERSISTENCE OF IMMUNE RESPONSE 7 YEARS AFTER ADMINISTRATION OF THE HPV-16/18 AS04-ADJUVANTED VACCINE TO WOMEN AGED 15–55 YEARS

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2 Department of Gynecologic Oncology, Poznań University of Medical Sciences, Poznań, Poland; 3 Department of Preventive Medicine, Poznań University of Medical Sciences, Poznań, Poland; 4 Praxis, Munich, Germany; 5 Charité Universitätsmedizin Berlin, Campus Mitte and Benjamin Franklin, Berlin, Germany; 6 GlaxoSmithKline Vaccines, Bangalore, India - 7 XPE Pharma and Science, Waver, Belgium; for GlaxoSmithKline Vaccines; 8 GSKaxoSmithKline Vaccines, Waver, Belgium

Objectives: The HPV-16/18 AS04-adjuvanted vaccine (HPV-16/18 vaccine, GlaxoSmithKline) has been shown to induce a robust immune response in women aged 15–55 years. We report the persistency of immune response and safety results from this study 7 years after first vaccine dose.

Methods: Females who received 3 doses of HPV16/18 vaccine at 15–55 years of age in an open, age-stratified study (NCT00196937) were invited for long-term follow-up (NCT00947115). Serological and cervicovaginal antibody responses were measured by enzyme-linked immunosorbant assay (ELISA).

Conclusions: At Month 84, in the according-to-protocol (ATP) cohort (N = 457), all baseline seronegative subjects were seropositive for anti-HPV-16 and all but 7 (one in the 26–45 years age group and 6 in the 46–55 years group) were seropositive for anti-HPV-18. Anti-HPV-16 and -18 antibody geometric mean titres (GMTs) remained several-fold higher than antibody levels after natural infection (Table) 7 years after the first dose. A decrease in serum antibody levels was observed with increasing age. In a total vaccinated cohort subset with 20 cervicovaginal secretion samples (CVS) available, HPV-16 and -18 antibodies were detected in CVS in 16 (80%) and 13 (65%) subjects, respectively. The vaccine had a clinically acceptable safety profile up to 7 years.

Persistent serological and cervical site specific antibody responses were demonstrated in women aged 15–55 years up to 7 years after the first dose.

Table. Serum anti-HPV-16/18 antibody levels at Month 84 (ATP baseline seronegative)

<table>
<thead>
<tr>
<th></th>
<th>GMT, EL. U/mL (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HPV-16</td>
<td>307.5 (256.2, 369.7)</td>
</tr>
<tr>
<td>Anti-HPV-18</td>
<td>119.8 (98.1, 145.9)</td>
</tr>
</tbody>
</table>

†From females (15–25 years) seropositive for anti-HPV-16/18 but negative for HPV-DNA (NCT00122681)

Study funded by GlaxoSmithKline Biologicals SA
**DISCISSION OF THE ADDERENCE LEVEL OF VACCINATION AGAINST HUMAN PAPILLOMA VIRUS (HPV) IN A UNIVERSITY POPULATION (SÃO PAULO – BRAZIL)**

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1. Academics of the fourth year and fifth year of medicine of the University of Santo Amaro (UNISA); 2. Prof. Dr. Head of the discipline of Obstetrics and Gynecology of UNISA.

**AIM:** Describe the vaccination adherence against the HPV of college students in a Brazilian University (Unisa) São Paulo - Brazil

**METHODS:** The dissemination of the HPV vaccine campaign was initially held in the academic conference at the university through educational lectures and later through digital reports to the inbox mail of all students. The goal of the campaign was to make immunization available at low cost. Three doses of vaccine were available to all students, both men and women, for a lower price (each dose is 150, totaling 450 reais) if compared with the prices offer of private offices (each dose is 300, totaling 900), and in Brazil vaccine is not yet available for everyone in the public health care.

**CONCLUSION:** Despite a contingent of more than 2,000 students of the university, only 290 (14.5%) attended the orientation lecture and 161 (55.5%) were vaccinated (149 women and 92.54% men and 12 - 7.45%). Of these amount 135 took the 3 doses, 156 took only two doses and one person took only 1 dose. These data shows that despite the disclosure by both lectures, and in digital report that was send for all students, the adherence was not significant, and the scenario is even worse when considering the male audience and in addition not everyone took the full prevention package of three doses, and not being completely immunized. It is concluded that is still needed major forms of dissemination among university students, especially among men, and is necessary to highlight the importance of taking the three vaccine doses at lower prices, while there is no available free vaccine at Brazilian public health care.

**CLINICAL TRIAL PHASE III B: EFFECT OF VACCINATION IN PATIENTS WITH RRP – ENROLLMENT DATA**

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2 Voice and Hearing Center, Medical Healthcom, Prague, Czech Republic

**Objectives:** Recurrent respiratory papillomatosis (RRP) is a rare disease that manifests by the recurrent growth of benign papilloma in the respiratory tract. Vaccines against human papillomaviruses are now commercially available. One commercial vaccine contains antigens of both HPV types which cause virtually all cases of RRP. In patients with RRP the level of HPV-specific antibodies is low and seems to increase only after numerous surgery procedures. The application of this vaccine to patients with RRP may stimulate increased levels of antibodies in the blood as well as on the surface of upper aero digestive tract where they neutralize viruses shed from new papilloma and thereby protect patient from spreading of the infection and facilitate the decrease of load of prog- eny infectious viruses.

**Methods:** In 2011 clinical trial phase III b (NCT 01375868) was started. Patients older than 18 years with the diagnosis of RRP were enrolled in the study. From all patients scraping of larynx and/or biopsy for HPV DNA detection and typing and blood for detection of anti-HPV antibodies was obtained. HPV detection and typing was done by means of PCR with GP5+/6+ bio primers and reverse line blot assay. Detection of HPV-specific antibodies was performed by direct ELISA with VLPs specific for HPV 6, 11, 16 and 18. Data on demographics, general risk factors for RRP, and risks related to HPV exposure were collected by a questionnaire.

**Conclusions:** To date 44 patients were enrolled. In seven patients RRP was detected for the first time, the remaining 37 patients had on average of 6 surgery interventions before enrollment. From 18 patients we have obtained both biopsy and smear, for two patients only biopsy was available. All biopsies were HPV positive, while 6 corresponding smears were HPV DNA negative. Altogether, 93% subjects were positive for anti-HPV antibodies. Thirty four percent of patients were positive for both anti-HPV 6 and 11 antibodies. No patient reported having condylomata acuminate in the past, only one patient reported diagnosis of condylomata acuminate in the sexual partner.

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**HPV INFECTION IN GIRLS VACCINATED IN THE CZECH REPUBLIC**

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**OBJECTIVES:** Currently two prophylactic HPV vaccines are commercially available to prevent HPV16/18 infection and associated lesions. The aim of the study was to assess markers of HPV infection in virginial and age-matched sexually active girls before vaccination and to observe the prevalence and spectrum of HPV types detected after vaccination.

**METHODS:** Seventy five virgins and 145 age-matched sexually active girls who signed informed consent were enrolled. Before the first dose and 1 and 3 years after the completion of HPV vaccination the cervical smear for cytology and HPV detection (not in virgins) and blood for assessment of anti-HPV antibody response were taken. The selection of vaccine type depended exclusively on vaccinated girls. Seroprevalence rates to HPV6, 11, 16 and 18 at enrolment were in virgins 30.7, 18.7, 5.3 and 2.7%. Corresponding percentages in sexually active girls were 26.9, 22.1, 13.1 and 4.8. Virgins 20-23 years of age were seronegative for all antigens. HPV DNA was found in 30.8% girls, HPV16/18 in 7.7%. Current HPV16/18 infection (DNA+/Ab-) was detected in 3.5%, persistent (DNA+/Ab++) in 4.2% and cleared (DNA-/Ab+) in 7.0% of sexually active girls. The prevalence of infection increased with age. At first FU 25% of virgins who started their sexual life and 41.8% sexually active girls were HPV DNA positive, at second FU it was 41.2% and 56.5%, respectively. During FU none of subjects acquired any of vacuccinal HPV type. Both vaccines induced antibodies to all vaccinal antigens, between 1st and 2nd FU antibody levels slightly decreased. In most girls seronegative for HPV31 and 33 at baseline these antibodies were elicited regardless of the vaccine used.

**CONCLUSIONS:** Results of the study demonstrate high prevalence of HPV infection in young women. High prevalence of antibodies in virgins suggests non-coital way of HPV infection transfer. The acquisition of HPV after onset of sexual life is very fast. Both current vaccines stimulate production of antibodies to HPV types 31 and 33 which are closely related to vaccinal types. Supported by IGA of Ministry of Health CR # NT/12372
HPV-VACCINATION COVERAGE FIVE YEARS AFTER INTRODUCTION: FIRST GERMAN POPULATION ESTIMATES FOR 14 TO 17 YEAR OLD GIRLS.

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**Objectives**

In Germany, vaccination against HPV infection has been recommended for girls aged 12-17 years since 2007. Early childhood vaccination coverage is generally high in Germany. However, gaps in uptake exist along social gradients for vaccination against measles and Hepatitis B and conclusive data were missing for HPV. The aim of this paper was to provide a general overview of the current status and determinants of HPV vaccination uptake in adolescent girls.

**Methods**

Analyses included data from 11 year-old female participants (n=1337) of the first follow-up (2009-2012) of the nationwide German Health Interview and Examination Survey for Children and Adolescents (KiGGS). KiGGS was a telephone survey using standardized interviews including questions for girls on the number of HPV vaccine doses and visits to a gynecologist. Parents were asked about their socioeconomic status (SES) and the girls’ participation in adolescent health check-up (J1). Descriptive statistical analyses of the HPV vaccination status (complete and incomplete series) with respect to social and demographic factors, and factors of health care utilization were performed. Factors associated with non-vaccination and their odds ratios were estimated by means of logistic regression analysis.

**Conclusions**

In adolescent girls the prevalence of vaccination with at least one HPV vaccine dose was 53.4% (95% CI 48.4-58.3). Coverage of three-doses of HPV vaccination was 37.7% (32.9-42.8). Vaccine uptake increases with age, was higher in girls with medium SES, with residence in eastern Germany and in those who participated in the J1. Multivariate logistic regression revealed a two-fold odds of non-vaccination for girls with high SES (Odds Ratio 2.15; 1.4-3.6) and for those who had not yet seen a gynecologist (OR 1.9; CI 1.3-2.8).

In Europe, HPV vaccine implementation varies largely. Successful programs with 80% coverage have been established in the UK, Portugal, Belgium and Denmark [1]; in Germany skepticism about the benefits of vaccination seems to outweigh recent scientific evidence.


VACCINATION AGAINST GYNECOLOGICAL CANCER: FROM NEW OPPORTUNITIES TO NEW HORIZONS.

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In countries with well established screening programs, concomitant implementation of HPV vaccination and screening are more effective in prevention of cervical cancer then screening alone, and also more cost-effective. In countries without organized screening HPV vaccination is most effective method of HPV-cancers reduction.

Australia introduced extensive, funded national HPV vaccination program with high coverage and broadest funded catch-up age in 2007. Vaccination was introduced in situation with organized and effective screening. Organized screening was implemented in Australia since 1991 using PAP-test. Participation rate is 60-86% aged 18-69 years old every 1-5 years. Resulted in cervical cancer morbidity reduced up to 50% by 2007. Main vaccination cohort are girls aged 12-13 years old. In 2007-2009 was funded catch-up vaccination for females younger then 26. In 2013 male vaccination was introduced. Three first years of HPV vaccination (2007-2009) resulted in 38% reduction of high grade cervical lesions. In 2011 genital warts near disappeared in young females.

In Russia cervical cancer morbidity and mortality is constantly growing, almost 40% of first diagnosed cervical cancer cases are in 3-4 phases, screening is opportunistic. Today HPV vaccination is not in National vaccination calendar. In 20 regions HPV vaccination is funded, but with low coverage and varies year to year. More then 200 000 doses of HPV vaccines were distributed in Russia. In 2008 school based HPV vaccination was introduced in Moscow region, 6850 girls (aged 12-13) were vaccinated, in 2012-2013 vaccination was continued (4000 girls were vaccinated). As a result incidence of genital warts in teenage girls in Moscow region reduced by 17%.

Taking into consideration high burden of disease, fast epidemiological spread of HPV there is urgent need in Russia to implement national funded HPV vaccination for girls 9-13 years old. Key success factor of HPV program is education of primary care HCPs, gynecologists and pediatricians. Russian and international experience is reviewed and adopted to local practice in national guidelines: “Cervical cancer prevention”.

SENSITIVITY OF THREE COMMERCIAL PCR-BASED ASSAYS FOR THE DETECTION OF HUMAN PAPILLOMA VIRUS IN A DILUTION STUDY

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**Objective**

Several PCR-based assays have been developed in the recent years for the detection and genotyping of Human Papilloma Virus (HPV). The assays have often been compared by performing HPV genotyping on formalin-fixed, paraffin-embedded tissue or on cytological material. Not much is known about whether these PCR-based assays are able to detect only a few HPV-infected cells on a background of several non-infected cells and if so, how many HPV-DNA copies have to be present per cell in order to get a positive test result.

**Methods**

A dilution study was conducted by mixing HeLa and SiHa cells with HEK293 cells in the following dilutions; 1/10 (one HeLa cell per 10 HEK cells), 1/100, 1/1000, and 1/10000. According to the manufacturer Hela cells contain approximately 50 copies of HPV 18 and SiHa cells 1-5 copies of HPV 16. After mixing, the cells were fixed with formalin and the mass was embedded in paraffin in order to imitate histological material. Three 10 µm sections were cut from each block and the DNA was purified using Qiagens QIAamp DNA FFPE Tissue Kit. The purified DNA was tested for HPV by means of three commercial assays; HPV sign genotyping test kit, INNO LIPA HPV genotyping extra, and LINEAR ARRAY HPV Genotyping test.

**Conclusion**

Results will be presented at the EUROGIN 2013. If it is possible to detect a very low copy number of HPV DNA among several non-infected cells using one of the methods mentioned above, the assay(s) might be capable of detecting HPV in a latent phase in epithelial cells of the human cervix.
ANALYTICAL EVALUATION OF APTIMA® HPV PERFORMANCE WITH SUREPATH LIQUID-BASED CYTOLOGY SPECIMENS USING THE APTIMA TRANSFER SOLUTION

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Objectives: The APTIMA HPV Assay (AHPV) is CE marked for use with SurePath (SP) specimens treated with FastExpress (FE) proteinase K and tested on the TIGRIS DTS System. A new treatment method was developed to improve detection and operational efficiency utilizing the APTIMA Transfer Solution (ATS), which consists of proteinase K reconstituted with a buffered formalin chelating reagent. The objectives of this study were 1) evaluate the robustness of the ATS treatment method, 2) evaluate the recovery of HPV E6/E7 mRNA from SP samples stored at 250°C out to 58 days as compared to the FE method, and 3) evaluate the reproducibility of the AHPV assay with ATS treated SP clinical specimens.

Methods: SP samples were prepared by spiking HPV infected SiHa or HeLa cells into pools of residual HPV negative SP specimens. For method robustness testing, the ATS incubation time (15 minutes), temperature (90°C) and volume (300µL) were varied by ± 5 minutes, ± 50°C and ± 50µL respectively. For the mRNA recovery evaluation, the spiked SP pools were stored at 250°C both neat and diluted 1:2.9 in STM, then tested out to day 42 (neat) and day 58 (dilute) with the AHPV assay, after ATS or FE treatment on the day of testing. AHPV Assay reproducibility was evaluated by testing 5 replicates of 30 HPV positive SP clinical specimens on two separate days, after ATS treatment on the day of testing.

Conclusions: Incubating samples for 10 to 15 minutes, at temperatures from 85°C to 95°C, or using 250 µL to 350 µL of ATS per sample did not impact the effectiveness of the ATS treatment, positivity was not affected. ATS treatment improved the recovery of HPV E6/E7 mRNA from SurePath samples, as compared to FE treatment, with greater than 20% increase in positivity observed. Agreement of the AHPV assay results was 100% for the ATS treated SP clinical samples between the two days of testing. These results indicate the ATS treatment is a robust method and improves detection of HPV mRNA from SP samples.

COMPARISON BETWEEN NESTED AND SINGLE-STEP PCR FOR HIGH-SENSITIVITY DETECTION OF HUMAN PAPILLOMAVIRUS

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Objectives: Human papillomaviruses (HPV) are the aetiological agents of certain benign and malignant tumours of the skin and mucosa, the most important being cervical cancer. In addition, the incidence of ano-genital warts and HPV-induced anal and oropharyngeal cancers is rising. Many different techniques have been proposed to detect HPV-DNA in cervical samples, but many lack the sensitivity required for certain types of clinical samples, e.g. FFPE samples (Formalin Fixed Paraffin Embedded). The nested PCR has been demonstrated to be one of the most suitable methods for such hard-to-analyse samples, but it suffers from being time consuming and prone to contamination. Here we present a new high-sensitivity one-step PCR assay for HPV detection and typing.

Methods: A group of 60 samples, including 29 FFPE samples, were tested for HPV infection and genotyped using two kits, AMPLIQUALITY HPV-TYPE HS and AMPLIQUALITY HPV-TYPE EXPRESS v2.0, that are based on nested and single-step PCR, respectively, and Reverse Line Blot (RLB) (AB ANALITICA). The assays were performed according to the manufacturer’s instructions, on DNA extracted with the QIAamp DNA mini kit (QIAGEN). Discrepent results were verified using the primer set described in Soderlund-Strand et al.

Conclusions: Concordant results were obtained in 51 cases. Seven samples were tested positive with AMPLIQUALITY HPV-TYPE EXPRESS v2.0 but gave a negative result with the nested PCR kit. It is noteworthy that six of these samples were found positive for high-risk oncogenic HPV genotypes (hrHPV). The only sample that gave a negative result with AMPLIQUALITY HPV-TYPE EXPRESS v2.0 and a positive result with the nested PCR, contained HPV 66. This study clearly shows the superior sensitivity of the new HPV detection and typing assay based on single-step PCR-RLB compared to the assay with nested PCR. This will ensure improved detection of hrHPV both in cytological and in hard-to-analyse clinical FFPE samples while reducing the amount of time needed and effectively preventing contaminations.
Objectives: Several studies have demonstrated that testing for HPV DNA after treatment of a high-grade intracervical neoplasia (CIN) with conisation is more sensitive than cytology control and with a very high negative predictive value. However, the specificity and positive predictive value for residual disease is limited. In studies on triage and primary screening the Aptima mRNA Assay has been shown to have comparable sensitivity but higher specificity than HPV DNA tests. Only few studies have been conducted evaluating the performance of a HPV mRNA test after treatment of a high-grade CIN.

Methods: 685 liquid-based cytology specimens (Thin-Prep, Hologic) received at the department of Pathology, Vejle Hospital, Denmark between October 2010 and December 2012 was included in this study. The samples were consecutive samples from women attending post-treatment surveillance, and the samples were taken between 2 and 24 months after conisation. All the samples were examined by cytology and by two HPV tests performed on the residual material according to manufactures instructions. The tests were Linear HPV DNA genotyping test (Roche) and the Aptima HPV mRNA Assay (Hologic). The age of the women included was 19-74 years, with a mean age of 34.1 years. The follow-up by histology and/or cytology were recorded. The sensitivities, specificities and predictive values were calculated using CIN2+/CIN3+ proven by histology as gold standard.

Conclusion: 29 of the women had histology-proven CIN2+ by follow-up and 20 women had CIN3+. The sensitivity for detecting CIN3+ for both assays was 100 % and for CIN2+ 93.1% for the Aptima Assay and 100% for Linear Array. The Specificity for detecting both CIN2+ and CIN3+ was significantly higher for the Aptima Assay than for Linear Array: 83.8% versus 63.6 % for detecting CIN2+ and 83.0% versus 62.7% for detecting CIN3+.

HC2 VERSUS COBAS HPV TEST IN SUREPATH SAMPLES FROM WOMEN AGED ≥30 YEARS WITH ASCUS

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Objectives: In Denmark, women aged ≥30 years with ASCUS are triaged to colposcopy based on reflex HPV testing. HC2 (Qiagen, Gaithersburgh, MD) is currently the most used HPV test for this purpose in SurePath samples in Denmark.Roche HPV test on the Cobas 4800 System (Roche, Pleasanton, CA) includes e.g., an internal control, and separate HPV 16 and 18 genotyping and therefore rendering this assay a candidate for triage of ASCUS positive samples. We compared the outcomes of HC2 and Cobas HPV test in women aged ≥30 years based on their SurePath samples showing ASCUS.

Methods: In total, 301 samples (204 from Herlev University Hospital and 97 from Hvidovre University Hospital) from women aged ≥30 years with ASCUS (median age: 43.8 years, range: 30-77) were collected in 10 ml of SurePath media. Eight ml were processed for liquid based cytology and HC2 analysis, while the remaining 2 ml reserved for later testing on the Cobas 4800 system. We calculated the proportion of overall agreement between the two assays, and retrieved subsequently histological data for women with discordant results from the Danish Pathology DataBank.

Conclusions: Overall agreement between HC2 and Cobas was 86% (260/301). Thirty-two (11%) women were tested positive on HC2 and negative on Cobas. Of these, histological results were available for 28: normal (20/28; 71%), CIN 1 (6/28; 21%), and CIN “Not Otherwise Specified” (NOS) (2/28; 7%). Nine women (3%) were tested negative on HC2 and positive on Cobas and their histology was available for two, with 1 normal and 1 CIN2 found. Among 198 women tested positive on Cobas, 128 (65%) were positive for 12 combined high-risk genotypes excluding HPV 16 and 18, followed by women positive for HPV 16 alone (n=32; 16%), and HPV 18 alone (n=14; 7%). Twenty-four (12%) women presented combinations of the 12 combined genotypes and HPV 16 and/or 18, while no HPV 16 and 18 co-infections were observed. In summary, Cobas and HC2 showed relatively good agreement in Danish women aged ≥70 years with ASCUS, and high-grade CIN was a rare finding in women with discordant HPV results.

INCIDENT HPV INFECTION AT 48 MONTH EXIT SCREEN AMONG WOMEN IN THE HPV FOCAL TRIAL

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Objectives: HPV FOCAL is the first North American population based randomized trial to evaluate primary high risk HPV DNA (HPV) screening (Digene hc2) plus liquid based cytology (LBC) triage of HPV positive women vs. LBC screening plus ASC-US triage with HPV within an organized screening program. The objective is to report incident HPV infection at the 48 month exit screen for women HPV negative at baseline.

Methods: Consented trial participants were women 25-65 yrs from the British Columbia Cervical Cancer Screening program, who were randomly assigned to receive either LBC (Control Arm) or HPV testing (Intervention Arm (IA)) at baseline. Women who are baseline HPV negative in the IA exit the trial at 48 months with both LBC and testing. At exit, those HPV positive or ≥ASC-US are referred to colposcopy.

Results: Of 9,553 women in the IA, 9,539 (99.9%) had valid baseline HPV results. 770/9,539 (8.1%) were HPV pos [<35 yrs: 334/1,862 (17.9%); 35-49 yrs: 318/4,457 (7.1%); 50+ yrs: 118/2,220 (5.3%)]] and 8,769/9,539 (91.9%) were HPV neg. Of women who have attended the 48 month exit screen and who were HPV neg at baseline, 49/1,435 (3.4%) were HPV pos [<35 yrs: 9/78 (11.5%); 35-49 yrs: 24/549 (4.4%); 50+ yrs: 16/808 (2%)]. Of the 49 HPV pos women, 42 have LBC and 9 have histopathology results available. 28 were LBC neg (histopathology: 2 normal, 2 CIN1); 4 ASC-US (1 normal; 9 LSIL (3 CIN3); 1 HSIL (1 CIN1).

Conclusions: Given the higher HPV prevalence among younger women, the incidence rate in this age group at exit is expected (<35 yrs: 11.5% vs. 35-49 yrs: 4.4% and 50+ yrs: 2%). Of women who have attended colposcopy, no high grade cervical lesions were detected by histopathology. Since the analytical sensitivity of hc2 is <100%, it is possible that infections classified as incident were not detected by hc2 at baseline. Correlation of hc2 screening results with those of other screening assays used at baseline and at exit is in progress to arrive at a more accurate 48 month incidence rate. Genotyping of the incident HPV positive specimens is also in progress.
### P 4-8

**ASSESSING THE ROLE OF A NOVEL PANEL OF mRNA BIOMARKERS FOR MANAGING WOMEN IN COLPOSCOPY.**

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**Objectives:** To evaluate the role of a novel panel of mRNA biomarkers for improving management of women presenting in colposcopy with abnormal smears. These biomarkers were developed as part of a FP7 programme grant AUTOCAT (201525).

**Methods:** 16 mRNA biomarkers and 12 HR HPV types (16,18,31,33,35,39,45,51,52,56,58,59) were simultaneously assessed using the OpenArray Technology (Life Technologies). Our study cohort comprises of 130 women who presented for their first visit to colposcopy following a series of abnormal smears, a smear sample was taken at first visit for HPV and biomarker analysis and in majority cases a biopsy was taken during colposcopy. Total RNA was extracted from PreservCyt cervical smear specimens, converted into cDNA and run on the Openarray platform. HPV data was correlated with COBAS 4800 (Roche Diagnostics) HPV results. Overall, 108 patients were diagnosed as having LSIL, (51), HSIL (24), ASCUS (23) and negative (8) on cytology. Of these, 35 were confirmed as CIN2+, 18 were CIN1 and 55 normal on histology. Using Cobas HPV test the prevalence of HPV in the entire study cohort was 56.4%. The prevalence of HPV detection on the OpenArray system was 50%. There was a good overall correlation between both methods for HPV detection (Kappa co-efficient 0.762, 95% CI: 0.598 to 0.926). For detection of CIN2+, the sensitivity was 92.1% and 91.4%, and specificity was 41.2% and 54.8% for Cobas 4800 HPV test and HR HPV detection on the OpenArray platform, respectively. Out of the 16 mRNA biomarkers analysed, there were different patterns of expression in CIN1 and CIN2+ groups relative to those patients who were normal on colposcopy. The majority of genes were upregulated and fold changes ranged from 41 fold increase in expression to 20 fold decrease in expression.

**Conclusion:** The OpenArray platform facilitates simultaneous HPV/DNA genotyping and biomarker analysis on the same samples thus cutting down on processing samples on different platforms.

### P 4-9

**PROSPECTIVE STUDY EVALUATING THE ROLE OF HPV mRNA-TESTING IN THE MANAGEMENT OF CERVICAL LESIONS CAUSED BY HR-HPV.**

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**Introduction and Objective:** Detecting HPV viral E6/E7 oncoproteins’ transcripts mRNA assay seems to be a useful marker of malignant progression. Aim of the study is to evaluate its performance and value in clinical management and follow-up of patients with HR-HPV persistent infection.

**Materials and methods:** From May 2011 to May 2013, 60 patients (age 24-56) referred to our colposcopy clinic for cervical lesions have been enrolled in the study.

All patients underwent traditional cytology, colposcopy and histology. Biopsies were done by punch or electrosurgical excision under colposcopic view. All patients were treated by LEEP after an observation period between 2 and 12 months. Nuclear acids detection was performed using NucliSENS EasyQ HPV Kit (BioMerieux) for mRNA and Hybrid Capture II (Digene) for DNA.

**Results and Conclusions:** All patients underwent colposcopy and mRNA detection assay. 54/60 had a positive HR-HPV DNA. A biopsy was done in 58 patients, of which 42 were positive for CIN II+. On the 60 patients all treated by LEEP 45 were diagnosed as CIN II+. We compared the results of mRNA assay and histology from the cone biopsies: 33/43 mRNA-positive patients had a CIN II+ histology and 5/17 mRNA-negative patients had a CIN I histology (correspondence between mRNA result and histology = 63%). On 22 patients referred with L-SIL/ASCUS cytology and a positive mRNA test, 13 had a worst histologic result at conization (CIN II+). mRNA and DNA assays were in accordance in 42/54 patients. Among 22 mRNA positive patients with a CIN II biopsy result, 13 had a definitive histology resulting as CIN III (59%); on 12 mRNA positive patients with a biopsy showing a CIN I, 7 (58%) had an histologic result of CIN II+ at conization. The most frequent HPV genotype detected in the group was HPV 16 (30/43 = 70% of the patients) either alone or as a co-infection (HPV 31 and 45). Our preliminary results underline the potential role of mRNA detection assay as a useful prognostic marker in identifying HR-HPV infected patients with a higher risk of malignant progression. Further longitudinal follow-up of our case series will help to better establish its performance and clinical value.

### P 4-10

**THE ROLE OF HPV VIRAL LOAD IN PATIENTS WITH ASCUS OR LSIL.**

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**Objectives:** To investigate the role of HPV viral load in the management of patients with ASCUS or LSIL.

**Method:** 843 patients who underwent loop electrosurgical excisional procedure due to ASCUS or LSIL which was documented on Papanicolau smear were included in this study. All patients underwent HPV test with a liquid hybridization assay at the time of Papanicolau smear and viral load was measured using light measurements by comparing the relative light units (RLU) of clinical samples with a positive control (PC), 1.0 pg/ml HPV 16 cutoff standard. Viral load was stratified into five groups using a log scale: negative (0-1 RLU/PC), low (1–10 RLU/PC), intermediate (10–100 RLU/PC), high (100–1000 RLU/PC), and very high (≥1,000 RLU/PC).

**Results:** Of 843 patients, viral load was 0-1 RLU/PC in 223 patients, 1-10 RLU/PC in 93 patients, 10-100 RLU/PC in 166 patients, 100-1000 RLU/PC in 227 patients, and >1000 RLU/PC in 124 patients. After LEEP pathology report showed normal cervix in 340 patients, CIN 1 in 184 patients, CIN 2 in 55 patients, CIN 3 in 227 patients, and invasive cervical cancer in 37 patients. The probability to have CIN or invasive cervical cancer increased gradually as viral load increase from negative to very high (P<0.001). In ROC curve, sensitivity and specificity was 74% and 66% in predicting CIN with the cutoff value of 20 RLU/PC.

**Conclusions:** Higher viral load imposed higher risk of CIN in patients with ASCUS or LSIL in Papanicolau smear.
COMPARISON OF INNOLIPA HPV GENOTYPING AND HYBRID CAPTURE-2 (HC2) ASSAYS FOR THE DETECTION OF HIGH-RISK HPV GENOTYPES AND CERVICAL INTRA-EPITHELIAL NEOPLASIA IN HIV-POSITIVE AFRICAN WOMEN: HARP STUDY

GILHAM C1, NGOU J2, CHIKANDIWA A3, SAWADOGO B4, KELLY H1, DIEDETOL M5, NAGOT N5, DELANY-MORET L5, MEDA N5, WEISS H1, MAYAUD P6, SEGONDY M7, on behalf of the HARP Study Group

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Objectives: To evaluate the performance of two HPV DNA assays, for the detection of high-risk HPV and CIN2+ among HIV-positive African women.

Methods: The HARP study enrolled HIV-positive women aged 25-50 in Burkina Faso (BF) and South Africa (SA). A stratified sampling strategy was used, with 2/3 of women on ART. Cervical HPV DNA was tested by Hybrid-Capture II (HC2, Qiagen) and genotyping was performed using Innolipa HPV genotyping Extra assay. A pap smear was collected for conventional cytology. Four-quadrant cervical biopsies were obtained among women with abnormalities detected by at least one test (HPV DNA, cytology, VIA or VILI) or by colposcopy.

Results: 632 and 625 women were enrolled in BF and SA, respectively. The distribution of CD4+ count (cells/µL) was similar in both sites: 68% with CD4+ >350 and 10% with CD4+ <200. HPV prevalence by HC2 was 44% among women in BF and 60% in SA. The prevalence of HR-HPV genotypes (the same 13 HR-HPV genotypes detected by HC2: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) detected by Innolipa was higher in both countries: 61% among women in BF and 80% in SA. Overall there was 73.8% agreement between the tests (Kappa Statistic = 0.47, p < 0.0001), which was higher in women with CIN2+ (91.3%) compared with women with <CIN2 (71.0%).

Of the 160 cases of CIN2+, there were 10 women who were Innolipa negative (for the 13 HR-HPV types) compared with 18 women who were HC2-negative. Therefore the Innolipa assay was more sensitive (93.8%), but less specific (34.2%) at identifying CIN2+ lesions compared with HC2 (89.4% sensitivity and 55.5% specificity).

Conclusion: There was good agreement between the tests especially in those with CIN2+. Comparisons between the performance of HPV DNA testing and cytology will also be presented.

P 4-12

COMPARISON OF INNOLIPA HPV GENOTYPING AND HYBRID CAPTURE-2 (HC2) ASSAYS FOR THE DETECTION OF HIGH-RISK HPV GENOTYPES AND CERVICAL INTRA-EPITHELIAL NEOPLASIA IN HIV-POSITIVE AFRICAN WOMEN: HARP STUDY

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Methods: The HARP study enrolled HIV-positive women aged 25-50 in Burkina Faso (BF) and South Africa (SA). A stratified sampling strategy was used, with 2/3 of women on ART. Cervical HPV DNA was tested by Hybrid-Capture II (HC2, Qiagen) and genotyping was performed using Innolipa HPV genotyping Extra assay. A pap smear was collected for conventional cytology. Four-quadrant cervical biopsies were obtained among women with abnormalities detected by at least one test (HPV DNA, cytology, VIA or VILI) or by colposcopy.

Results: 632 and 625 women were enrolled in BF and SA, respectively. The distribution of CD4+ count (cells/µL) was similar in both sites: 68% with CD4+ >350 and 10% with CD4+ <200. HPV prevalence by HC2 was 44% among women in BF and 60% in SA. The prevalence of HR-HPV genotypes (the same 13 HR-HPV genotypes detected by HC2: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) detected by Innolipa was higher in both countries: 61% among women in BF and 80% in SA. Overall there was 73.8% agreement between the tests (Kappa Statistic = 0.47, p < 0.0001), which was higher in women with CIN2+ (91.3%) compared with women with <CIN2 (71.0%).

Of the 160 cases of CIN2+, there were 10 women who were Innolipa negative (for the 13 HR-HPV types) compared with 18 women who were HC2-negative. Therefore the Innolipa assay was more sensitive (93.8%), but less specific (34.2%) at identifying CIN2+ lesions compared with HC2 (89.4% sensitivity and 55.5% specificity).

Conclusion: There was good agreement between the tests especially in those with CIN2+. Comparisons between the performance of HPV DNA testing and cytology will also be presented.

P 4-13

APTIMA TRANSFER SOLUTION PRETREATMENT OF SUREPATH SAMPLES USING HEAT AND ENZYME BEFORE TESTING BY APTIMA HPV COMPARED TO COBAS 4800 HPV

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3 Thunder Bay Regional Health Sciences, Thunder Bay, CAN - 4 Hologic/GenProbe, San Diego, USA

Objectives: Testing of Liquid-based Pap (L-Pap) samples for high risk HPV has led to increased sensitivity for detection of cervical precancerous lesions and improved strategies for managing and treating patients. HPV DNA and mRNA assays are available, however performance with most assays have been compared using PreservCyt L-Pap samples; few have evaluated SurePath L-Pap samples. We are conducting a prospective study to evaluate the clinical performance of the APTIMA HPV (APTV) assay and the APTIMA HPV 16, 18/45 Genotype assay on the TIGRIS and PANTHER instruments with SurePath and digene HCV platforms treated with the APTIMA Transfer Solution (ATS) collected from women attending colposcopy clinics. For this interim analysis, APTIMA results are compared to cobas 4800 HPV test results.

Methods: SurePath samples were tested with the cobas 4800 HPV DNA test (Roche) using a published protocol. Two aliquots of the SurePath samples, 0.5mL and 1.0mL, were transferred into tubes containing APTIMA specimen transport media and then treated according to the ATS kit protocol (protease K and heat). After treatment, samples were tested with the AHPV assay on the TIGRIS and PANTHER instruments (Hologic/Gen-Probe). Agreement between AHPV and cobas 4800 HPV and between TIGRIS and PANTHER, as well as Kappa statistics, were calculated. Clinical performance estimates will be calculated when biopsy information is available. To date 162 women enrolled in the study have complete cobas 4800 HPV and AHPV results.

Conclusions: Overall agreement between cobas 4800 HPV and AHPV/TIGRIS for the ATS-treated 0.5mL sample was 90.7% (Kappa 0.815) and ATS-treated 1.0mL sample was 90.1% (Kappa 0.813). Agreement between AHPV/PANTHER and cobas 4800 HPV was similar for the ATS-treated 0.5 mL sample (88.3%, Kappa 0.767) and the ATS-treated 1.0 mL sample (91.3%, Kappa 0.827). Agreement was 94.4% (Kappa 0.889) for AHPV performance between the PANTHER and TIGRIS platforms for both treatment strategies. ATS pretreatment of SurePath samples shows very good overall agreement between AHPV and cobas 4800 HPV results.
OBJECTIVE: High-risk human papillomavirus (HPV) DNA detection provides high sensitivity but low specificity for moderate-grade cervical intraepithelial neoplasia or worse (CIN2+) identification. The objective was to evaluate the mRNA testing efficacy for predicting CIN2+ in women with atypical squamous cells of undetermined significance (ASCUS) and HPV DNA detection.

METHODS: 107 patients with ASCUS and HPV 16/18/31/33/45 DNA detection were included. Endocervical samples were tested with NucliSENS-EasyQ1 HPV E6/E7 mRNA-assy (Biomerieux) after RNA extraction with Rneasy Plus minikit (Qiagen). Patients were referred to colposcopy and mRNA results were compared with histology results within 7 months after DNA detection. mRNA sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and their relative 95% confidence intervals (95% CI) for CIN2+ were calculated (Epida 3.0) considering the CIN2+ histological diagnosis as the disease endpoint and “gold standard”. Women who had undergone colposcopy without a biopsy were grouped with benign results and CIN1.

CONCLUSIONS: colposcopy was not performed in 2 patients and biopsy was not conclusive in 3 cases. CIN2+ was diagnosed in 22/102 (21.6%) women. mRNA detection was considered inhibited in 3 cases. Clinical utility of mRNA assay for CIN2+ detection (% 95% CI) was: SE (85.7, 68.4-100), SP (44.9, 33.2-56.5), PPV (29.5, 17.2-41.8), NPV (92.1, 82.2-100). If only mRNA positive cases were referred to colposcopy, colposcopies would be reduced by 38.4% losing 14.3% of CIN2+. In conclusion, this mRNA-assy can serve as a triage test for ASCUS in case of HPV 16/18/31/33/45 DNA detection because of its high NPV.

THE POTENTIAL USE OF DIRECT E6/E7® HPV WHOLE-CELL ELISA™ FOR TRIAGING PATIENTS WITH HIGH-RISK HPV POSITIVE ASC-US OR LSIL CYTOLOGY

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Objectives: We have developed a robust, high-throughput Direct E6/E7® HPV Whole-Cell ELISA™ to detect human papillomavirus (HPV) E6/E7 oncoproteins in HPV-associated precancers and cancer. To assess the potential use of this test for triaging patients with high-risk HPV positive (HPV+) ASC-US or LSIL cytology, we compare the Direct E6/E7® HPV Whole-Cell ELISA™ assay performance to that of cytology, HPV DNA and biopsy results.

Methods: In on-going prospective study, 3372 residual liquid-based cervical cytology samples (ThinPrep®) with ASC-US or LSIL and HPV+ results were obtained from a screening population at CytoCheck Laboratory, LLC, Kansas, USA. We performed Direct E6/E7® HPV Whole-Cell ELISA™ with our non-type specific monoclonal anti-HPV E6 and E7 antibodies. Assay positivity was defined using a pre-defined cut-point (OD450=0.396). The positivity rate of Direct E6/E7® HPV Whole-Cell ELISA™ was measured in all samples and compared to the histological grade of available cervical biopsies (n=416) using Fisher’s exact tests.

Conclusions: In 3372 samples tested, 1761 and 1099 had ASC-US and LSIL respectively. While all cases were HPV+, only 865 (26%) were Direct E6/E7® HPV Whole-Cell ELISA™ positive. To date, colposcopically-directed biopsy results are available from 416 cases: 7 CIN3 (2%), 33 CIN2 (8%), 324 CIN1 (74%), and 52 negative (12%). Direct E6/E7® HPV Whole-Cell ELISA™ was positive in 71% of CIN3+. For <CIN3, the specificity of Direct E6/E7® HPV Whole-Cell ELISA™ was 79%. These results suggest that Direct E6/E7® HPV Whole-Cell ELISA™ may provide improved specificity for disease detection in women with HPV+ ASC-US or LSIL. To further confirm the histology results, available biopsy slides are under adjudicated pathology review. Re-analysis of the test results with the consensus histology results will also be presented.

COMPARISON BETWEEN TWO COMMERCIALLY AVAILABLE METHODS FOR MOLECULAR DETECTION OF HIGH RISK HPV.

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Objective: High-risk genotypes of human papillomaviruses (HR HPV) are etiologically linked to cervical carcinomas. HPV DNA testing provides an alternative approach to cervical screening and patient management. The objective of this study was to compare the results of two commercially available tests for high risk HPV genotyping.

Methods: Genital samples from 274 patients were collected using Digene STM (Qiagen, Hilden, Germany) and tested with CLART HPV (Genomica, Madrid, Spain) and Abbott RealTime High Risk HPV test (Abbott Molecular, Des Plaines, IL, USA). The CLART HPV (Genomica, Madrid, Spain) is based on a polymerase chain reaction (PCR) for HPV DNA amplification followed by a low density microarray. This method allows the detection and identification of 20 high risk e 15 low risk HPV types. The Abbott RealTime High Risk HPV test (Abbott Molecular, Des Plaines, IL, USA) is a real-time PCR assay based on concurrent individual genotyping for HPV-16 and HPV-18 and pooled detection of 12 other HPV’s (HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66 and HPV-68).

Conclusion: Detection rates and correlations are presented in Table 1. The agreement between the methods was 86.1%, 38 samples showed discordant results. We found no case of samples detected in CLART and not detected in Abbott RealTime PCR. Discrepant results may be caused due different limits of detection between the methodologies and competition when more than one HPV type is present, considering that CLART HPV is able to detect low risk HPV. We concluded that Abbott RealTime High Risk HPV had a higher rate of detection of high risk HPV DNA.

### Table 1. Comparison between Abbott RealTime High Risk HPV and CLART HPV.

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<th>CLART (HPV Microarray)</th>
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<td>Detected</td>
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<td>Abbott (HR-HPV)</td>
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<td></td>
<td>Detected</td>
<td>80</td>
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<td><strong>Total (%)</strong></td>
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**THE ROLE OF HPV GENOTYPING, P16/KI-67 DUAL STAIN AND KOILOCYTOSIS IN THE PREDICTION OF CERVICAL LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS**

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**Objective:** Low-grade squamous intraepithelial lesions (LSIL) may progress to high-grade cervical lesion or even more frequently persist or regress. To distinguish precancerous changes that require treatment from spontaneously resolving disease, additional analyses are needed. We evaluated the association of HPV genotyping, p16/Ki-67 dual stain and koilocytosis and their prognostic significance in LSIL lesions.

**Methods:** Samples were collected from 155 women referred to colposcopy. Cytology diagnosis of LSIL and koilocytosis were reviewed by two observers on conventional Pap smears. A reprocessed cytology slide was stained using the p16/Ki-67 dual stain cytology assay and HPV genotyping conducted for high risk HPV DNA positive cases. Follow-up period was 6 to 72 months. Patients with at least two consecutive negative Pap smears were classified as regression, while ASCUS or LSIL and histological diagnosis of CIN1 recorded as disease persistence. Progression was considered a histologically proven diagnosis of CIN2 or higher.

**Conclusions:** Most of the LSIL cases in our study regressed or persisted. We found that 13.8% of all cases progressed to CIN2 or CIN3 lesions and those associated with HPV 16 genotype often developed higher lesions. HPV 16 was found in 16.3% of all cases and more frequently in younger women, under 30 years. p16/Ki-67 dual immunocytochemical staining was positive in 36.1% of all cases and the positivity was more often associated with HPV 16 genotype presence compared to HPV non-16 genotypes. Moreover p16/Ki-67 positive dual stain cases was correlated to higher progression rate (30.8%) when compared to negative cases (4.3%). When the effect of specific HPV genotype infection and cytology features was analyzed, HPV 16 positive women without koilocytosis appeared to have an increased risk of CIN2+ development compared to patients with LSIL, HPV non-16 genotype and koilocytic morphology. A combination of HPV genotyping, immunocytochemical evaluation of p16/Ki-67 and koilocytic morphology can be useful in the prediction of the clinical outcome in women initially diagnosed as LSIL in Pap test cytology.

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**EVALUATION OF THE NOVEL ANYPLEX™ II HPV28 GENOTYPING ASSAY FOR DIAGNOSIS AND TYPING OF HPV FROM ARCHIVAL CLINICAL SAMPLES**

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**Objectives:** There is an increasing need of diagnosis and genotyping of human papillomavirus (HPV) in formalin-fixed paraffin embedded tissue (FFPE), to follow up clinical samples from precursor lesions and neoplasia particularly in cervix but also in head, neck, and ano-genital lesions. In this study we have evaluated a new multiplex assay, Anyplex™ II HPV28, which detects 28 genotypes of HPV and is based on a modified real-time PCR and melting curve analysis. The method has previously been validated on liquid based cytology specimens, however, not on any archival tissue. Anyplex™ II HPV28 was compared to a reference method which is a type-specific real-time PCR that detects 14 HPV genotypes.

**Methods:** In the study, 99 FFPE samples from patients with a clinical suspicion of HPV infection at the Department of Pathology, Örebro University Hospital were included. The majority of the samples were cervical lesions (61%; 60/99) and penile carcinomas (20%; 20/99), and the remaining were a mix of vulvar, vaginal, anal, tonsil, larynx, skin and lymph node metastasis. Anyplex™ II HPV28 (Seegene) detects HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 69, 70, 73, 82 and betaglobulin in 2 multiplex reactions. All tests were run on a CFX96TM Real-time PCR System (BioRad). For each run 3 positive controls, i.e. plasmid constructs of all HPV types, were included. The reference method detects HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and betaglobulin and were analysed on the 7900 HT Real-Time PCR System (Life Technologies).

**Results:** When only comparing the genotypes that were detectable by both assays, 63% of the samples were HPV positive with Anyplex™ II HPV28 compared to 67% with the reference method. Including all genotypes, Anyplex™ II HPV28 detected HPV in 72% of the samples. Both methods detected HPV 16 in 30/99 and HPV 18 in 8/99 (identical samples in both methods). Notable was that Anyplex™ II HPV28 detected HPV 56 in 11/99 samples compared to 1/99 with the reference method. Discrepancy analysis using additional genotyping method is pending.

**Conclusions:** Anyplex™ II HPV28 appeared effective for genotyping of HPV in archival clinical samples and could be used in a clinical setting.

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**OPTIMIZATION OF REPORTING GENOTYPES WITH OVERLAPPING HYBRIDIZATION PATTERN BY INNO-LIPA HPV GENOTYPING EXTRA**

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**Objectives.** INNO-Lipa HPV Genotyping Extra (Innogenetics) is a frequently used genotyping test. If probes with specific hybridization pattern partially overlap between various HPV genotypes, some genotypes can not be excluded to be present in a certain sample and they are reported in parentheses as possibly present. This form of reporting is prescribed by the manufacturer but is rather puzzling for the clinician requesting the test. Especially when high risk HPV types are showing possible overlap, the results send back to the clinic can be confusing. The aim of this study was to evaluate an added value of reporting possible overlapping hybridization patterns.

**Methods.** HPV genotyping was performed on 189 clinical samples and 20 external quality controls using INNO-Lipa HPV Genotyping Extra (Innogenetics) and Full Spectrum PCR HPV Amplification and Detection/Genotyping System by Lab2Lab Diagnostic Service (GenoID). The comparison of INNO-Lipa results with GenoID-method was performed using 2 different reporting strategies, one including possible overlap and another one excluding it.

**Conclusions.** For high risk HPV genotypes, HPV 39, 52 and 68 should be reported according to the manufacturer’s instruction as possibly present based on overlapping hybridization with other HPV types. Assuming actual presence of HPV 39, 52 and 68, the frequency of detection of these genotypes plotted against the total prevalence of high risk HPV types is respectively 7%, 35% and 1% higher as compared to the reporting without possible overlapping hybridization. The discordance between INNO-Lipa and GenoID increased by 12%, 21% and 8% for HPV 39, 52 and 68 respectively if possible overlapping hybridization was considered. Especially striking results were found for HPV 52 as it was reported in 53% of the clinical samples if possible hybridization pattern was considered. In external quality controls, the HPV 18-positive sample was reported as possibly harbouring HPV 39 if overlapping hybridization was used. Summarising, major differences were found in prevalence of HPV 39, 52 and 69 depending on reporting with or without possible hybridization.
HPV E6II GENE EXPRESSION AS AN INDICATOR OF CANCER SEVERITY IN INTRAEPITHELIAL CERVICAL LESIONS OF UTERINE CERVIX – A PILOT STUDY.

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Objectives: The etiological role of HPV in cervical cancer has been proved. Integration of the HPV genome into a host cell DNA leads to the consistent but deregulated expression of the viral E6 and E7 oncoproteins. This is a key factor for progression from low-grade cervical lesions to high-grade lesions and invasive cervical cancer. The aim of our study was to analyze the expression levels of HPV E6/E6II and E7 genes in cervical neoplasia of different grade (CIN1/CIN2/CIN3), and in normal cytology samples.

Methods: The analysis involved 35 specimens obtained from women with abnormal cervical smears. The studied group involved 10 low-grade squamous intraepithelial lesions (CIN1) and 15 high-grade lesions (CIN2 and CIN3), as well as normal cytology samples. Genomic DNA and total RNA were isolated from the cytological material obtained from the cervical canal surface. HPV genotyping was performed using RealLine HPV 16/18 kit. The expression analysis was performed using gene-specific primers and SYBR Green fluorescent dye in real-time PCR assay.

Conclusions: The presence of HPV16 DNA was found in 65.71% patients, including also normal cytology samples. In all studied samples the expression of E61 was revealed, with the increased level in 12 (34.3%) patients. The expression of E6II was observed in 10 (28.6%) studied samples, and it was increased in all those cases. The expression of E7 was found in 20 (57.1%) studied samples, and increased in 14 (70%) patients. Significant negative correlation was observed between the amount of input HPV16 DNA and the levels of E61 and E6II expression. There were no statistically significant differences in gene expression levels between the studied groups (CIN1 vs. CIN2/CIN3 vs. normal cytology). We found statistically significant differences in CIN2/CIN3 group, with the higher expression of E6II as compared with E6I. We suggest that the expression level of E6II gene might be used as an indicator of cervical cancer severity, in patients with high-grade cervical neoplasia, but these observations need to be confirmed in larger patient cohort.

P 6-2

P16 IN ENDOMETRIAL CANCER AND ITS POSSIBLE CORRELATION TO HUMAN PAPILLOMAVIRUS.

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Objective: The objective of this study was to investigate if diffuse p16 (surrogate marker of HPV) can be detected in endometrial cancers and in normal endometrial tissue.

Methods: The data is based on p16 positivity of biopsies from the Patobank at Aarhus University Hospital. Forty-one cases of endometrial cancers and a control group of 42 endometrial biopsies on benign indication were tested. Subsequent the biopsies were all evaluated by the same pathologist for the presence of p16. The p16-coloring had four possible outcomes: no reaction, <10%, 10-50% and >50%.

Conclusion: A border between focal and diffuse p16 colouring at 10% made a convincing result of diffuse p16 being more present in endometrial cancer, with a Risk Ratio of 9.03 CI 95%[3.54;23.06] and an Odds Ratio of 68.45 CI 95%[17.02;275.23] (p<0.000). If the border between diffuse focal p16 was set at 50% none of the controls had a positive outcome for diffuse p16. Diffuse p16 with >50% colouring was significantly overrepresented in endometrial cancer with a Risk Ratio of 3 CI 95%[2.12;4.25] (p<0.000).

These preliminary data suggest that diffuse p16 and thereby HPV is more present in endometrial cancers. These findings suggest that HPV could be an oncological factor in endometrial cancer development. Previously, diffuse p16 has been used to differentiate between endometrial and cervical cancers hence cervical cancers are of HPV origin. These finding suggest that this method should be revised.

P 6-3

CIN REGRESSION IS HIGHER IN SMALL HPV16+ LESIONS AND IN HPV16- LESIONS OF PATIENTS THAT CONSISTENTLY USED CONDOMS


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Objectives: High risk Human Papilloma Virus (hrHPV) is a necessary cause for Cervical Intraepithelial Neoplasia (CIN) and is the most common sexually transmitted agents worldwide. However, only 30% progress to cancer and 6-50% regress spontaneously. Despite of this, high grade CIN (CIN2-3) is usually treated with cone excision and women are exposed for the risk of side effects. Different HPV genotypes have different carcinogenic potential, and HPV16 is the most carcinogenic with the highest risk for CIN3 to progress into cervical cancer.

Methods: A prospective population-based cohort study of 145 consecutive women aged 25-40, with first-time onset diagnosis of CIN2-3 in colposcopy-directed biopsies was carried out. The aim was to investigate whether presence (HPV16+) or absence (HPV16-) of hr-HPV genotype HPV16 in punch-biopsies, semi quantitative scoring of immunohistochemical biomarkers (CD4, CD8, CD138, CD25, p53 and p53), combined with clinical features like sexual history, contraception type, smoking habits, parity or family history of cervical dysplasia or cancer, are correlated to regression of CIN2-3. The biopsy-cone interval was median 16 weeks. Regression was defined as CIN1 or less in the cone.

Conclusions: The results showed that 54/145 (37%) were HPV16+ and 91/145 (63%) were HPV16-, with no significant differences in regression rate for the groups. The HPV 16+ group had significant lower levels of p53 (p=0.002) and CD4 (p=0.03) positives as compared to the HPV16- group, but no significant differences were shown for p56, CD8, CD138 or CD25 positives. In the HPV16+ group, lesion size < 2.5 millimetres showed higher probability for regression (p=0.03). For the HPV16- group, consistent condom use was an independent variable for regression rate, 13% (10/79) to 73% (8/11), p<0.0001.
**P 6-4**

**METHYLATION OF LIQUID BASED CERVICAL CYTOLOGY INFECTED WITH HUMAN PAPILLOMAVIRUS TYPE 16 DNA ACCORDING TO THE DEGREE OF CERVICAL PATHOLOGY**

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**Background:** Development of cervical cancer is causally related to infection with high-risk human papilloma virus (hr-HPV). Although hrHPVs can be detected in many women, progression of a hrHPV-positive premalignant lesion to invasive cancer is rare. Epigenetics refers to the mechanism that regulates the cell type or tissue specific transcription or gene expression levels without altering the DNA sequences, through biochemical modifications such as the addition of a methyl group to cytosines, and post-translational modification of histone proteins. The aim of this study was to evaluate the presence of hypermethylated genes according to the degree of cervical disease.

**Material and Methods:** Study subjects with HPV 16 DNA positive were 122 women attending Seoul St. Mary’s Hospital from January 2009 to July 2011. Cervical cytology positive for HPV 16 were normal, CIN1, CIN2, CIN3, and cancer. Using quantitative bisulfite- pyrosequencing, we measured the methylation of PAX1, CADM1, ADCYAP1 and MAL genes of cervical scrapings.

**Results:** Cervical scrapings were used for detection of the methylation in normal (n=29), CIN 1 (n=29), CIN2 (n=4), CIN3 (n=42), and cervical cancer (n=18). We observed that 4 genes (PAX1, CADM1, ADCYAP1, MAL) were more frequently methylated in cervical cancer (83.3, 77.8, 88.9 and 72.2 %) than in normal cytology (0, 3.4, 3.4 and 6.9%). ROC curve demonstrated that methylation of the 4 genes discriminated between high grade cervical lesions (<CIN 3) and low grade cervical lesions (< CIN3). The estimated sensitivities of these 4 genes for detecting high grade lesion were 100, 96.6, 96.6 and 93.1% (p=0.0001). And the estimated specificities of that were 83.3, 77.8, 88.9 and 93.1%(p=0.0001). Additional ADCYAP1/PAX1 methylation analysis on the HPV16-positive women increased the sensitivity to 88% with a specificity to 96.7%(P<0.001).

**Conclusion:** Epigenetic events play a significant role in the development and progression of malignancy. When DNA is methylated in the promoter region of genes, when transcription is initiated, genes are inactivated and silenced. In our study, methylation of the PAX1, CADM1, MAL and ADCYAP1 is significantly associated with the development of ≥ CIN3.

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**P 6-5**

**DYNAMIN INHIBITORS AS ANTI-CANCER AGENT IN CERVICAL CARCINOMA**

Jeong-Won Lee, Yoo-Young Lee, Aera Yoon, Jin-Yong Park, Tae-Hyun Kim, Jun-Kuk Choi, Cheol Hun Choi, Tae-Joong Kim, Duk-So Bae and Byoung-Gie Kim

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**Purpose:** Cervical cancer is still the second most common cancer of women worldwide and nearly 1/3 of patients will die due to disease progression and recurrence. However, the therapeutic efficacy of the chemotherapty is very limited and new treatment target is needed. Dynamin II is a 96 kDa GTPase enzyme which has a key role in cytokinesis including cell division, migration, and endocytosis. Recently, dynamin II is reported to be associated with not only human papilloma virus (HPV) infection on the host cell but the prognoses of cervical neoplasms including cervical intraepithelial lesions and cervical cancer which suggest dynamin II may be a new treatment target for the cervical cancer treatment.

**Methods:** In this study, we performed tissue microarray analysis to compare the expression levels of dynamin II and pathological findings which are associated with poor prognosis in early cervical cancer. And, in vitro study, we investigated whether dynamin II inhibitors (siRNA, MiTMAB, OcTMAB, Dynasore, DD-6 and DD-11) could affect cell survival and invasion of various cervical cancer cell lines. To evaluate the role of dynamin II in cell proliferation, we performed MITT assays with Hela and SiHa cells after incubation with various doses of MiTMAB, OcTMAB. To confirm the apoptosis after MiTMAB and OcTMAB treatment, active caspase-3 ELISA and FACS were performed. And to identify the role of dynamin II in cell invasion, we evaluated the secretion of MMP-9 after dynamin II inhibitor treatment by ELISA assay.

**Results:** Normal cervical epithelium did not show the expression of dynamin II and higher expression of dynamin II was associated with larger tumor size (higher expression vs. lower expression; 3.6cm (0.9-10.5) vs. 3.3cm (0.5-7.5), p= 0.05) and the number of pelvic lymph node involvements in patients with early cervical cancer (higher expression vs. lower expression; 0 (0-7) vs. 0 (0-2), p= 0.194). In vitro, we found that MiTMAB and OcTMAB had a growth-inhibiting effect at 2 or 3 day after treatment in cervical cancer cells. Active caspase-3 expression and Annexin V intensity were increased by MiTMAB and OcTMAB. And the secretion of MMP-9 was increased after dynamin II inhibitor treatment. Similar results were obtained with Dynasore and DD-6.

**Conclusion:** These data revealed that dynamin II modulates cervical cancer cell survival, apoptosis, and invasion and suggest that dynamin II could be a key treatment target for new strategies in cervical cancer treatment.

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**P 6-6**

**HPV E6/E7 MESSENGER RNA AS A MARKER FOR NON-RECURRENTNESS OF CIN AFTER CONISATION?**

Dr Lilli Pandiani-LABCO ; Dr Nicolas Rouyer DIAG ; Dr Christophe Sattornnet DIAG

**Objective:** To evaluate the clinical utility of an HPV RNA assay for predicting disease recurrence in a test of cure setting, up to 8 years after loop electrosurgical excision procedure.

**Method:** The analysis described relates to a total of 59 liquid based cytology samples (Surepath, BD) taken up to 8 years after treatment of CIN2-3 from women attending routine cervical cytology follow-up in first quarter of 2013. Residual samples were obtained from a Cytology laboratory and tested using the Aptima HPV Assay (AHPV, Hologic) and the PAX-AMS assay (SRL Diagnostics, Tokyo, Japan). The sample inclusion criteria was that the women had a loop electrosurgical excision in the past eight years, between 2004 and 2012 after treatment of CIN2-3. The average age was 42 years (from 23 to 58).

**Results:** From the 59 samples were tested, results can be divided in two groups:

- Group 1: 2 samples (5 %) were cytology and AHPV positive, concordant with recurrence rate found in the literature (5 to15 %).
- Group 2: 57 negative samples with APTIMA HPV assay and cytology negative for 53 of them. Out of these 57 negatives, 2 samples showed a “positive margin” from conisation done in 2009 and 2010, respectively, and 2 other samples showed morphological signs of HPV infection in LBC.

**Conclusion:** These data revealed that HPV E6/E7 messenger RNA was significantly associated with a lower risk of recurrence when compared to the HPV DNA assay.
LEUCINE-RICH IMMUNOGLOBULIN-LIKE REPEATS (LRIG) 1, 2 AND 3 IN CERVICAL NEOPLASIA.

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Background: Cervical neoplasms; invasive cancer and intraepithelial neoplasia (CIN), are sexually transmitted infections, with human papillomavirus (HPV) infection as the main etiological agent. Defining factors that are correlated to increased risk, diagnosis, prognosis and other clinical features are important.

Methods: 129 invasive cervical cancers in stages IB to IV, 47 cases of high grade CIN, 59 cases of low-grade CIN and 64 biopsies from normal epithelium were consecutively recruited. The cervical biopsies were evaluated for LRIG expression, and a total of 15 other relevant biological tissue markers (tumor markers) in invasive cancer and CIN. A structured questionnaire, and serum estradiol and progesterone were included.

Results: In early stages of invasive cancer LRIG 1 expression correlated to a favorable prognosis (90% vs. 64% survival), while the reverse was true for LRIG 2 expression (60% vs. 87% survival). Low expression of LRIG 1 and high for LRIG 2 indicated a very poor prognosis (26% vs. 66%). LRIG 3 expression had no impact on prognosis. Smoking and high serum progesterone correalted to absence of LRIG 1 expression. In CIN both LRIG 1 and LRIG 2 expression increased with increasing severity of the lesion. There was a correlation between LRIG 3 expression and HPV infection as well as three tumor suppressors (Rb, p53 and p16) and use of progestogenic contraceptives, whereas LRIG 2 correlated negatively to Rb. Both LRIG 1 and LRIG 2 correlated to expression of tumor suppressor FHIT.

Conclusion: There seems to be biological roles for LRIG 1, LRIG 2 and LRIG 3 in HPV-associated cervical neoplasia. In invasive lesions LRIG 1 is associated with suppression, LRIG 2 with progression of the tumor, while the role of LRIG 3 remains obscure. In CIN LRIG expression correlate to a number of events associated with outcome.
High grade lesions progress to cancer by the expression of E6 and E7 oncogenes integrated in the cell DNA. Early diagnostics of progression to cancer is needed. Biosensors have potential advantages over other methods of cancer detection by increased assay speed, multi-target analyses, and reduced costs. We report the synthesis of novel HPV-L1 peptides: HPV-C3.2 ((YIK)2K-Ahx-C) and HPV-C6 (C-Ahx-SPINNTKPHEAR) with sequences recognized by antibodies against HPV-16. Peptides have cysteine in the amino acid sequence, its thiol side chain is responsible of chemisorption and self-assembly of peptides on polycrystalline gold surfaces.

**Objective.** To determine electrochemical characteristics of modified polycrystalline gold electrodes with peptides, design and develop an immunosensor for HPV oncoproteins.

**Methods.** Peptide synthesis was made by orthogonal strategy solid phase peptide synthesis. Amino acids were pre-activated with DCC and HOBt. The N–deprotection was performed. Solvent wash with DMF, DCM and IPA interspersed was used after coupling and deprotection. Reactions were monitored by a ninhydrin assay. Peptide cleavage and side-chain deprotection were performed. Peptides were precipitated using cold ethyl ether, resulting solids were dissolved. Crude peptides were analyzed by RP-HPLC with a XDB-C18 column. Cleaved crude material was purified. Characterization was done by RP-HPLC and mass spectroscopy. Polycrystalline gold electrodes were polished to a mirror-like finish. Peptides were self-assembled on bare gold electrodes by its immersion in 1 mM of peptide solution in methanol. Infrared measurements were used to characterize the peptide self-assembly on screen-printed gold electrode modified, with a Thermo Nicolet spectrometer and OMNIC software.

**Conclusion.** Novel peptides HPV-C3.2 and HPV-C6 were self-assembled on two different gold surfaces by Au-S linkages. With an increment in the current density to the process Ru(NH3)6+3/+ compared with the bare gold by the pre-concentration of the charge, favored for the presence the side chain of the amino acids from the peptides, confirmed by FT-IR. Improvement in electronic transfer processes on surfaces modified with these peptides allowed estimation of parameters to detect antigens to develop an immunosensor for HPV oncoproteins.
SIGNIFICANCE OF P16 IMMUNOSTAINING IN POSTMENOPAUSAL WOMEN WITH ATYPICAL SQUAMOUS CELLS

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Background: Interpretation of postmenopausal Pap smears is one of the most difficult areas in gynecological cytopathology. Degeneration associated with hyperchromatic crowded groups, parabasal cells with organophilic cytoplasm or variations in nuclear size may often be misinterpreted as squamous atypia or even more severe lesion. In the present study we wanted to evaluate the significance of p16 immunostaining in postmenopausal women with atypical squamous cells.

Methods: The study included 36 asymptomatic postmenopausal patients (37 smears) with the initial cytological diagnosis of ASC-US or ASC-H on the conventional Papanicolaou smears. The smears were decolorized and immunostaining for p16(Ink4a) was applied. In 32 patients (33 smears) tissue biopsy and histological examination was performed. Four ASC-US cases had cytological follow up only.

Results: Positive p16 reaction was found in 22 patients (23 smears) and negative in 14 of them. The histological diagnoses in p16 positive cases were: CIN1 (3 patients), CIN2 (3 patients), CIN3 (11 patients /12 smears), AIS (1 patient) and invasive squamous carcinoma (2 patients). Two p16 positive patients with initial negative history had CIN3 after two years. CIN3 was detected in two p16 negative patients and normal in eight. Four p16 negative patients without histological examination had normal cytological follow up.

Conclusion: p16 is a useful marker for detection of clinically significant cervical lesions in asymptomatic postmenopausal women with equivocal cytology results.

DETECTION OF CHROMOSOMAL CHANGES IN REGIONS 3Q26 (HTERC) AND 8Q24 (MYCC) IN PRECANCERS AND CARCINOMA OF THE UTERINE CERVIX

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Objectives: Characteristic marker of malignant tumors is chromosomal instability, either numeric or structural, which has a prognostic and predictive impact in many tumor diseases. The amplification of gene hTERC (3q26) and gene MYCC (8q24) was detected in cervical intraepithelial neoplasia and carcinoma of the uterine cervix. These specific chromosomal changes can predict progressive premalignant lesions and cervical carcinomas.

Methods: From November 2008 to January 2013 we studied copy number changes of the chromosomal region of hTERC and MYCC genes using the HPV-interphase fluorescent in situ hybridization (HPV-FISH) in cytological smears of precancers and carcinoma of the uterine cervix in the Czech Republic.

Results: We assessed correlation of incidence of genetic abnormalities with clinical and cytological tumor stages. 26 women were currently in our file with carcinoma of the uterine cervix. The amplification of hTERC a MYCC genes were detected in stage IA1 in 50 % (1 of the 2 patients), in stage IB1 in 86 % (12/14). Amplification was found in all patients of stage IA2 (2 women), stage IB2 (3 women) and stage IIb with lymph node metastases (5 women). Women with proven amplification of gene hTERC had positive lymph vascular space involvement in 67 %. In the group of 6 women with cervical carcinoma in situ the gain of hTERC and MYCC was found in 67 % (4/6). In the group of 20 women with cervical intraepithelial neoplasia the chromosomal abnormalities were detected in 50 % (2/6 - CIN I, 3/4 - CIN II and 6/10 - CIN III).

Conclusions: The principal aim of our study is to optimize the investigative methodology and to acquire information about chromosomal changes which are related with carcinogenesis of cervical cancer. The detection of the amplification of hTERC and MYCC genes could be predict progression of premalignant lesions and cervical carcinomas and could be used as a new genetic marker.

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IMMUNOME PROFILING: A POWERFUL TOOL FOR DIAGNOSTICS AND THERAPEUTICS

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OBJECTIVES: The immune system is a key mechanism to fight diseases. Profiling of the activity of the antibodies (binding to antigens) enables us to develop novel diagnostic tools for variety of diseases and conditions. We have developed a workflow to identify and monitor the global activity of immunoglobulins. Currently, a small number of autoantibodies is used in diagnostics of autoimmune and other diseases. The immune profiling, however, would be to rapidly assess the spectrum of different health conditions that have a reflection in the antibody profiles.

Methods: Exposure to infections accounts for 17% of human cancers, with human papillomavirus (HPV) being one of the most prevalent ones. HPV infection causes cervical cancers as well as a significant proportion of cancers of the vulva, vagina, penis, anus, and oropharynx; together these HPV-related cancers account for >5% of all human cancers.

The antibody response to HPV is virus subtype-specific, and profiling of the global antibody activity would contribute to determining of the spread of type-specific HPV infections in populations, monitoring of the effect of HPV vaccines in inducing protective antibodies, but most of all to evaluation of oncogenic threat of the infection. The antibody response to HPV is complex since infection and disease lead to distinct virus subtype-specific antibody responses. At present there is no clinically useful serological assay of HPV exposure or susceptibility.

CONCLUSIONS: Clinical validation of the set of peptide mimotopes enabled to identify a group of peptide biomarkers that would be clinically applicable as diagnostic biomarkers for precancerous lesions. Overall, these results demonstrate the power of the developed technology.
A RANDOMISED CONTROLLED TRIAL OF RESPONSE TO SELF-SAMPLING FOR HPV IN PERSISTENT NON-RESPONDERS IN NEWCASTLE UPON TYNE, UK (THE SHINE TRIAL)

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Objectives: The only previous UK trial on self-collected samples in non-responders to date was carried out in London. This showed a very low response rate of 6.4% in contrast to 27-39% in other European studies and 9-20% in an Italian study, depending on the level of intervention. The London trial uptake was surprisingly low and it is unclear whether this is typical of the England as a whole or an anomaly. The repetition of the trial in a second English population aims to determine whether this is the case.

Methods: This was a randomised (1:1) controlled trial of 6000 women identified as not having responded to a first invitation, a first reminder letter, plus the first non-responder card sent to their GP. The Intervention group was sent an invitation to collect their own sample for HPV testing and the comparator group was sent a further cytology. Women were given 90 days to respond to the invitations. The main outcome measures were (1) percentage of women attending for cervical cytology compared with those returning a self-sample HPV test or attending for cytology subsequent to receiving the kit and (2) percentage of those testing positive for HPV who attended further investigation

Findings: The rate in the intervention group was 9.2% within 90 days; 13% of these tested positive for high risk HPV. The response rate of the comparator group and the further rate of attendance for follow-up cytology in the intervention group will be available shortly and will be presented.

Conclusions: On preliminary analysis, these data appear similar to those found in London, suggesting that this strategy may be less effective in England than in other European countries.

P 7-2

CAN WOMEN WHO HAVE HAD A HYSTERECTOMY DUE TO NON-MALIGNANT INDICATIONS BE EXCLUDED FROM CERVICAL CANCER PREVENTION ACTIVITIES?

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Objectives: Due to the low risk of cervical cancer in well-screened women, the last invitation to organized cervical cancer screening is offered at the age of 60 in Sweden. However, approximately 20% of all women exiting the screening program at age 60 have not attended for at least ten years. A large proportion of the women who did not attend prior to reaching the upper age limit for organized screening have had a hysterectomy and were advised not to take further smears according to Swedish guidelines. As a part of the nationwide Swedish audit of cervical screening, we analyzed the occurrence of subsequent vaginal and cervical cancer among women who had been hysterectomized for reasons other than cancer/dysplasia. The objective was to determine if women who have had a hysterectomy for non-malignant indications can be safely excluded from targeted follow-up of non-attenders.

Methods: We followed a cohort of 7,267,210 Swedish women from 1982-2005, of which 174,863 were totally hysterectomized after 1965. Information on date of birth, date of surgery, and diagnosis at surgery of these women were obtained from the National Hospital Discharge Register. Information on diagnosis of vaginal or cervical cancer was available from the National Cancer Registry for the years 1982-2005. “Cervical cancers” diagnosed after hysterectomy possibly represents misclassified vaginal cancers or misclassified type of hysterectomy, and were therefore included in the present study.

Conclusions: Among the 174,863 women, with a total hysterectomy, 25 were diagnosed with a vaginal cancer and 13 were diagnosed with a “cervical” cancer, while this was true for 12,476 of the non-hysterectomized women The incidence of vaginal- or cervical cancer in hysterectomized women was 1.58/100 000, and 11.54/100 000 in non-hysterectomized women, resulting in an age-standardized relative risk of 0.11 (95% CI=0.08-0.15). We conclude that women hysterectomized for benign reasons are at very low risk for developing cancer, and can be excluded from further invitations. Therefore the proportion of insufficiently screened women to be targeted after the age of 60 or older is probably not much higher than 10%.

P 7-3

P16INK4A/Ki67 VS. HPV TESTING FOR TRIAGE OF LSIL

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Objectives: In the Eurogin 2012 we presented an evaluation of the new generation DNA and RNA tests, like the Roche cobas® HPV, the Abbott Realtime HR HPV, Cervista™ and the Aptima HPV, comparing it with Hybrid Capture 2. The high prevalence of HPV limits the usefulness of HPV DNA testing when deciding how to manage LSIL, however the study showed a better specificity of the new DNA and RNA tests, comparing to HC2, due to the lack of cross-reactivity with low risk types. In the same samples we performed the CINtec Plus dual-stain for p16INK4A and Ki67 and therefore we pretend to evaluate the usefulness of this technique, when compared to HPV testing.

Methods: A maximum of 100 consecutive PreservCyt samples diagnosed with LSIL were selected from the routine screening at LAP. All results were compared with routine cytology and histology data generated for that sample. Information regarding the evaluation sample, patient information and data regarding other clinical samples relating to that patient were taken from the laboratory database to allow the clinical significance of the results to be assessed. All data stored for the evaluation was anonymised. The gold standard is histological confirmed cervical intraepithelial neoplasia (CIN) grade 2+ or 3+. Sensitivity and specificity of each of the HPV screening tests was calculated based on disease defined as CIN 2+. The CINtec Plus dual stain is performed in a new slide from the same vial or in the primary slide diagnosed with the LSIL.

Conclusions: The positivity rate was 81% for HC2, 71% for cobas® and Cervista, 69% for Abbott Realtime and 65% for Aptima HPV. The increase in specificity was evident, without loss of sensitivity. In our perspective, this finding could re-launch the debate around HPV testing for triage of LSIL. We are collecting the data obtained with the use of CINtec Plus in order to see if this benefit is even more evident for this technique. From a technical point of view, the CINtec Plus, currently lacks automation and still remains with some subjectivity when reading the results. Until assay automation is achieved, the use of CINtec Plus will be limited in large settings, when compared to completely automated HPV testing.
IMPROVING ATTENDANCE TO THE CERVICAL CANCER SCREENING PROGRAM IN NORWAY: PILOT STUDY FOR SELF-SAMPLING

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Background: There is a continuous decline in the 3-year coverage of the Norwegian cervical cancer screening program among women 25-29 years of age. Before the cancer screening program started (in 1995), the three year coverage (1992-1994) was 82%, and today (2009-2011) it has declined to 53% [1, 2]. The latest report from the Cancer Registry of Norway (2012) shows a small but alarming increase in cervical cancer over the last 10 years in women under 35 years of age [3], which underlines the need for interventions to improve coverage of the screening program. Two different types of self-sampling devices will be tested; a dry brush (Evalyn Brush, Rovers Medical Devices B.V., Oss, Netherlands) and a lavage based (Delphi Screener TM, Delphi Biosience).

Objectives: to assess whether self-sampling is a feasible alternative to cell samples taken by the doctor in cervical cancer screening.

Methods: Each month approximately 2000 2nd reminders are sent out to women in the Oslo Region, and ~1300 do not respond to this reminder. From those women due to receive a 2nd reminder, we randomly selected 800 for self-sampling, 600 within age-groups of 25-34 and 35-49, and 400 in 50-69 years. The remaining women received the standard 2nd reminder letter. Of total 800 women in the self-sampling group, half received the dry-brush and the other half the lavage-based, together with a questionnaire to assess user-friendliness and acceptability of the different self-sampling devices. Based on previous studies, we conservatively estimate a response rate of 30% [4]. Both devices will be analyzed for hrHPV by Hybrid Capture2 (HC2) High-Risk HPV DNA Test® (QiAGEN, Gaithersburg, MD, USA) and HPV genotyped (CLART, Genomica, Madrid, Spain).

Conclusions: We are in the process of receiving self-sampling devices and questionnaires. Follow-up for the self-samples will be closed by 01.10.2013. Acceptability of the lavage-based and dry brush devices will be assessed by performing logistical uni- and multivariate regression analysis. The difference between the two self-sampling devices will be quantified by using appropriate statistics. HPV results on two different sampling devices will be summarised.

COMPARISON OF THE PAPILLOCHECK AND INNO-LI PA HPV GENOTYPING EXTRA ASSAYS FOR HPV DETECTION AND TYPING.

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Objectives: A need for accurate genotyping of human papillomavirus (HPV) infections is becoming increasingly important to understand the pathogenesis of cervical cancer and for epidemiologic studies of HPV infections worldwide.

The aim of this study was to compare the performances of the PapilloCheck® (Greiner Bio-one) with the INNO-LiPA HPV genotyping Extra test (Innogenetics) for HPV detection and typing.

Methods: A total of 116 liquid-based cytology samples (38 with normal cytology, 52 with atypical squamous cells of undetermined significance ASCUS, 25 with moderate to severe dysplasia) were tested by the two assays. Extraction of HPV DNA was performed using the Nuclisens® EasyMag extraction kit on a EasyMag extractor (Biomérieux). Comparison analysis was limited to the HPV genotypes common to both assays.

Results: Agreement between the 2 assays was obtained for 88 (75.9%) samples: 46 (39.6%) showing complete concordance and 42 (36.2%) showing partial concordance with at least one HPV type identified by the two assays. A discrepant result was observed in 28 (24.1%) samples. The HPV positivity rate was 75% with PapilloCheck and 81.7% with INNO-LiPA, after restriction of the analysis to high-risk HPV types, the positivity rate was 61.2% and 76.7% respectively. The rate of multiple HPV infections was 37.1% with PapilloCheck and 41.4% with INNO-LiPA. Among women with normal cytology, HPV Prevalence was 52.6% by PapilloCheck and 86.8% by INNO-LiPA and for high-risk HPV, it was 44.7% and 71% respectively. Among women with ASC-US HPV prevalence was 78.8% by PapilloCheck and 82.7% by INNO-LiPA, and in women with moderate to severe dysplasia HPV prevalence was 92.0% by PapilloCheck and 96.0% by INNO-LiPA. Eleven samples negative by PapilloCheck and positive by INNO-LiPA were analyzed by sequencing: 5 were found positive for HPV and six could not be amplified for sequencing.

Conclusion: The two tests appears as valid methods for detecting and genotyping HPV subtypes. The discrepant results were mainly observed in cervical specimen with normal cytology. Most of the discrepancies seem to be due to a difference in sensitivity for HPV detection.

CAN HPV TEST REPLACE CYTOLOGY IN CERVICAL CANCER SCREENING?

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Objectives: Improvement of existing methods of early detection of cervical pre-cancer and cancer (cytology Pap test and HPV DNA test) is a key tool to reduce the cervical cancer-related mortality. The aim of this study was to reveal the false-negative results of cobas HPV Test in detection of HPV-related cervical lesions.

Methods: 88 women >25 years of age with abnormal cervical cytology (B&G, TriPath, USA) were investigated with cobas HPV test (cobas HPV test, Roche). The additional smears were prepared from the same samples for immunocytochemical (ICC) investigation with double staining for p16 (INK4a) and Ki-67 (CINtec PLUS cytology kit). Colposcopy and diagnostic biopsies were performed in all cases.

Results: 92 Pap tests were investigated with the following cytological diagnoses: 56 ASC-US, 10 L-SIL and 26 HSIL. All smears with H-SIL (26/26) and L-SIL (10/10) were positive for p16 (INK4A) and Ki-67, while in ASC-US — in 5 of 56. Comparison of ICC results of H-SIL and L-SIL with histological and IHC dates revealed their full coincidence. HPV DNA was detected in 24 out of 26 H-SIL cases, in 9 out of 10 L-SIL, in 4 out of 5 ASC-US. All women had histologically confirmed CIN of different grades. The total number of false-negative results of HPV testing was 4 (H-SIL-2, L-SIL-1, ASC-US-1) out of 89 (4,5%). In these 4 cases HPV DNA was not detected while p16 (INK4a) and Ki-67 were positive.

Conclusion: obtained results show that despite the high sensitivity of molecular methods they have disadvantages. False-negative HPV test results might be explained by existence of other HPV types that are not included in the system or by the presence of other cancerogenic factors that are involved in cervical cancer development. Our data indicate that combination of liquid base cytology, ICC and HPV testing with genotyping for HPV 16/18 gives greater opportunity to identify precancerous conditions.
CYTO-HISTOLOGICAL CONCORDANCE IN LSIL AND HSIL PAP SMEARS - 5 YEARS EXPERIENCE

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Objectives: To evaluate the concordance between PAP test results (LSIL and HSIL) and histological findings after cervical conization.

Material and Methods: Retrospective study including all women with LSIL (n=109) and HSIL (n=225) in PAP test seen in Cervical Pathology Unit, Faro’s Hospital, between 2006 and 2010, who was submitted to cervical conization. Each group was evaluated for: Age, parity, age of first sexual intercourse, number of sexual partners, histopathological findings after cervical conization and follow-up.

Results: On LSIL group age ranged between 17 and 59 years (average 33.46 years, STD 8.76%), 30.28% were nulliparous and 69.72% multiparous, average age of first sexual intercourse was 17.60 years (STD 2.32), average number of sexual partners was 2.90 (STD 1.81) and histopathological results revealed CIN 2 or higher (CIN 2+) in 58.72%, CIN 1 in 31.19% and no cervical dysplasia in 10.08%. 4.59% have needed a second intervention later and 23.85% have abandoned follow-up.

On HSIL group age ranged between 17 and 80 years (average 38.83 years, STD 10.08), 17.33% were nulliparous and 82.67% multiparous, average age of first sexual intercourse was 17.50 years (STD 2.90), average number of sexual partners was 5.56 (STD 10.02) and histopathological results revealed CIN 2+ in 81.78%, CIN 1 in 14.67% and no cervical dysplasia in 3.56%. 14.22% needed a second intervention later and 20.89% have abandoned follow-up.

The difference between the 2 groups revealed statistical significance regarding multiparity (p≤0.01), average number of sexual partners (p<0.01), risk of CIN 2+ for HSIL PAP smear (p<0.01) and needs of a second intervention (p≤0.01). The risk of CIN 2+ was 23.06% higher for HSIL group.

Conclusions: PAP test is considered the goldstandard screening for malignant and premalignant lesions of cervix. LSIL is usually associated with HPV cellular changes and CIN 1, while HSIL include CIN 2, CIN 3 or carcinoma in situ. In the present study, as expected, HSIL showed a high concordance with CIN 2+ found in histopathological results (81.78%) and only 3.56% had no cervical dysplasia. Although LSIL has showed a significantly lower risk, it was unexpected to find such a high rate of CIN 2+ (58.72%), showing the need for study, follow-up and eventually treat these women. Multiparity and the number of sexual partners appear to be risk factors for malignancy.

CERVICAL CANCER SCREENING IN A PUBLIC HEALTH CENTER: A 5 YEARS EXPERIENCE.

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OBJECTIVES: Our goal was to analyze cytological results in women undergoing gynecological examination for different reasons at a public health hospital and evaluate their performances

METHODS: A retrospective analysis of all the cytological and histological samples were carried out from January 2006 until January 2011 (54224 slides in total).

CONCLUSIONS: 90% of all results were negative. As abnormal results we were able to find: 2.93% ASCUS, 0.48% ASCH, 3% LSIL, 1.18% HSIL, 0.82% AGC, 0.03% AIS and 0.23% of Invasive Carcinoma. 2482 hystological samples were taken and we confirmed 280 cases of CIN1, 171 patients with CIN2, 268 cases of CIN 3 and 220 cases of Invasive carcinoma. Although its obvious limitations, cytology can still be an effective screening method.

PAP SMEAR AND CERVICAL HISTOLOGY AFTER SHORT TIME REPEAT HIGH-RISK HPV TESTING

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Objectives: Women with persistent high-risk HPV infection as well as women with an abnormal Pap smear run an increased risk of cervical carcinoma. The relationship between Pap smear and cervical histology was examined in women with short time persistent high-risk HPV infection.

Methods: A cohort of 5000 women, 36-65 years old, who had not participated in organised Pap smear screening for 6 years or longer were offered self-sampling of vaginal fluid at home, followed by high-risk HPV testing (HC2). Women with HPV infection were offered admittance to a gynaecological surgery, after around 2 months, for follow up with colposcopy, cervical biopsy, a Pap smear and a repeated HPV test (hpVir). In all, 35% (1752/5000) of invited women chose to participate and 6.5% (114/1752) were HPV positive. 99 women (compliance 87%) accepted to attend a gynaecological surgery, after around 2 months, for follow up with colposcopy, cervical biopsy, a Pap smear and a repeated HPV test (hpVir). In all, 35% (1752/5000) of invited women chose to participate and 6.5% (114/1752) were HPV positive. 99 women (compliance 87%) accepted to attend a gynaecological surgery, after around 2 months, for follow up with colposcopy, cervical biopsy, a Pap smear and a repeated HPV test (hpVir).

In all, 35% (1752/5000) of invited women chose to participate and 6.5% (114/1752) were HPV positive. 99 women (compliance 87%) accepted to attend a gynaecological surgery, after around 2 months, for follow up with colposcopy, cervical biopsy, a Pap smear and a repeated HPV test (hpVir). In all, 35% (1752/5000) of invited women chose to participate and 6.5% (114/1752) were HPV positive. 99 women (compliance 87%) accepted to attend a gynaecological surgery, after around 2 months, for follow up with colposcopy, cervical biopsy, a Pap smear and a repeated HPV test (hpVir).

Conclusions: Short time repeat HPV testing seems to be a considerably more specific screening method for detection of pre-malignant CIN2-3 lesions than primary Pap smear screening.
A PILOT STUDY TO INVESTIGATE THE EFFICACY OF FIBRIN SEALANT (TISSEEL®)
IN LOOP ELECTROSURGICAL EXCISION PROCEDURE (LEEP)

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Objectives: The objective of the current study was to evaluate the efficacy and feasibility of fibrin sealant (Tisseel®) in loop electrosurgical excision procedure (LEEP) for cervical intraepithelial neoplasia (CIN 2 or 3).

Methods: We designed a single-blinded prospective randomized study in 40 consecutive women undergoing LEEP for biopsy-confirmed CIN 2 or 3 at Uijeongbu St. Mary's Hospital, Korea. Informed consent was obtained from all participants. 2 ml of fibrin sealant (Tisseel®) was applied to the uterine cervix of 20 women right after LEEP surgery (treatment group). We evaluated abdominal pain, vaginal bleeding, vaginal discharge and impairment in daily living during postoperative week 1 for the treatment group and the control group was 0.3±0.80 days vs 1.7±2.36 days (p=0.015) and 0.9±1.37 days vs 1.5±2.25 days (p=0.015) respectively. 14.6% (11/77) of the women reported severe symptoms. The mean duration of moderate to severe vaginal bleeding and impairment in daily living during postoperative week 1 for the treatment group and the control group was 0.3±0.80 days vs 1.7±2.36 days (p=0.015) and 0.9±1.37 days vs 1.5±2.25 days (p=0.015) respectively. There was no difference in the bipolar rate in women who did not receive fibrin sealant treatment (control group).

Conclusions: The percentage of young patients treated for cervical intraepithelial neoplasias (CIN) has been increasing, thus it is very important to define patients in high risk for relapse. The aim of this study was to establish any possible association of persistent human papillomavirus (HPV) infection, and smoking, with relapse of CIN.

Methods: A prospective clinical study was performed at the Clinic of Obstetrics and Gynecology in Nis, with the study group comprising the first 35 patients with disease relapse after conization and the control group consisting of 30 patients with more than one year after treatment without relapse. HPV typhotyping was done at the Laboratory for Molecular Biology and Cytogenetics of the Clinical Centre Nis using polymerase chain reaction (PCR).

Results: Women in the control group were more commonly non-smokers (56.6% vs. 40%); comparison with the study group showed a significant difference (p>0.05). We did not find any statistically significant difference (p>0.05) for the distribution of tobacco cigarettes of the study and control subjects. Patients with recurrences were more commonly HPV-positive compared to controls (68.57% vs. 66.6%) (p<0.05). In the study group, HPV-positive patients who were smokers suffered recurrence with more advanced stages (CIN III and MIC) (p<0.01). In non-smokers, the severity of recurrence grade was not statistically correlated with HPV positivity. It thus seemed that smoking could be regarded as a co-carcinogen, acting as one of the promoters of HPV infection.

Conclusion: Persistent HPV infection and smoking associated with HPV infection were demonstrated to be of statistical significance for CIN relapse. Smoking as independent etiologic factor was not significantly associated with CIN relapse.
Anticancer effect of bee venom toxin and melittin in uterine cervical cancer cells

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Objectives: We studied whether bee venom inhibits cancer cell growth through enhancement of death receptor expressions in the human cervical cancer cells, CaSki and C33A.

Methods: To determine the effect of bee venom and its major component, melittin on the uterine cervical cancer cells, apoptosis is analyzed by tumor assay and apoptotic gene expression. To investigate whether bee venom and melittin can inactivate nuclear factor kappa B (NF-κB), we assessed NF-κB activity in vitro.

Results and conclusions: Bee venom inhibited the growth of CaSki and C33A cervical cancer cells by the induction of apoptotic cell death in a dose dependent manner, but apoptosis was more induced in CaSki cells than in C33A cells. Expression of DR3 and DR4 was increased in both cells, but expression of them in CaSki was more than increased than C33A. Expression of DR downstream pro-apoptotic proteins including caspase-3 and Bax was concomitantly increased, but NF-κB activity and the expression of Bcl-2 were inhibited by treatment with bee venom in CaSki and C33A cells. However the effects were more significant in CaSki cells. Combination treatment of expression of TVEAK and TRAIL (a ligand of DR3 and DR4) and bee venom synergistically inhibited the cervical cell growth with further down regulation of NF-κB expression. Moreover, deletion of DR3 and DR4 by small interfering RNA significantly reversed bee venom induced cell growth inhibitory effects as well as NF-κB inactivation.

Bee venom induces apoptotic cell death in cervical cancer cells through enhancement of DR3 and DR4 expression and inhibition of NF-κB pathway, however, the sensitivity of bee venom is different by the status of p53 in the cervical cancer cells. These results suggested that it may be applicable as an anti-cancer agent for cervical cancer, especially p53 wild type cancer.

Clinical presentation, treatment and outcome of vaginal intraepithelial neoplasia

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Objectives: Vaginal intraepithelial neoplasia (VAIN) is a rare disease among women. Despite its low incidence between 0.2 and 0.3 per 100,000 women VAIN is an important disease due to the risk of progression to an invasive carcinoma of the vagina. This study was conducted to characterize the patients, their symptoms and the clinical presentation of the disease among a fairly large group of women with VAIN.

Methods: The medical records of 65 women were evaluated for this study. Follow up data were available for only 33 patients.

Conclusions: The mean age was 61 years (range, 32 – 89 years). Most patients (51%) were admitted with abnormal cytology findings. Of all 65 women 35% had VAIN 3, 28% had VAIN 2 and 31% had VAIN 1. Most lesions (55%) were found in the upper third of the vagina. 42% were multifocal lesions. 69% of all women were high-risk (HR) HPV positive and 11% were HPV negative. 32% had a CIN earlier or at the same time as the VAIN, mostly CIN 3 (66%). 66% had a hysterectomy in the past, often because of high grade CIN.

Most cases of VAIN were treated with CO2-Laser-vaporisation. Other treatment methods were local excision, partial or complete colpectomy. There were no follow up data available for 48% of the women. 46% had a confirmed relapse of the disease while 6% had follow up visits with no signs for relapse. There was no significant correlation between patient age and HR-HPV positivity or patient age and multifocality of the lesions. Multifocal VAIN was less frequently HPV positive than single lesions. Multifocal VAIN was also less likely to be cured than single lesions when only women without pathological findings after therapy were considered as cured. However, both of these results were not significant. There was also no significant influence on the relapse rate by simultaneous CIN, mode of therapy or history of hysterectomy.

The only significant influence on the relapse rate that could be identified was the HR-HPV status. HR-HPV positive VAIN was significantly more likely to relapse than HR-HPV negative VAIN (p = 0.003, unifactorial ANOVA for women with confirmed cure of disease; p = 0.043, unifactorial ANOVA for all women without confirmed relapse).

This study shows that VAIN can be difficult to treat especially if it is HR-HPV positive. Abnormal vaginal cytology, hysterectomy and CIN in the past are important risk factors for the development of the disease.

Human papillomavirus in intraepithelial neoplasia and carcinoma of the vulva

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Introduction: Cancer of the vulva is a rare type of cancer. The exact cause of vulvar cancer is still unclear; the persistent infection of HR HPV is a described risk factor. In Portugal, data from 2006 register around 134 cases of vulva and 30 of the vagina, represented a total rate of 2.45 compared with the a world rate of 0.97 and an European rate 1.49.

AIM: Detection of DNA HPV in samples with vulvar lesions.

Methods: We study 89 samples of females recurrent to the gynecology consult from Oncology Portuguese Institute with a mean age of 67 years (range 22-92 years) with histological result confirmed. Real time PCR (SYBR Green), using SPF primers, was used to evaluate HPV presence. HPV positive samples were genotyped using Microarrays (PapilloCheck) or INNO-LI PA (Innogenetics). As Negative, positive and internal control were used MRC5, SiHa and B-globin, respectively.

Results: HPV DNA was detected in 57.3% (51/89) of the samples against 42.7% (40/89) of negative results. HPV prevalence by Histological diagnosis were 53.2% (42/79) in vulvar squamous cells carcinomas (SCC), 50% (1/2) vulvar intraepithelial neoplasia (VIN I/II) and 100% (8/8) in condilomas. Simple infection were represented in 64.3% (18/28), where the most prevalent types detected were HPV16 44.4% (8/18), HPV6 22.2% (4/18) and HPV39 11.6% (2/18). In all co-infections have at least one HPV HR type.

Conclusions: In conclusion, our present study suggests a prevalence of HPV 53.2% in vulvar squamous cells carcinomas, 50% in vulvar intraepithelial neoplasia and 100% in condilomas, where the most prevalent types were HPV16. This proportion would be higher than that found in literature for the vulvar SCC (53.2% vs 40%), but similar in condilomas (100% vs 99%).
Objectives: Persistent infection with HPVs is a major risk factor for the development of anal cancer. The detection rates of HPV DNA in anal cancer tissue specimens range from 70 to 100%, with HPV16 and HPV18 being the most frequently detected HPV types. Although disruption and integration of HPV genome into the host genomic DNA is considered to be a key event in the progression towards invasive cervical cancer, little is known about this event in anal carcinogenesis. The aim of this study was to determine the prevalence and distribution of HPV types and HPV16 genomic variants in patients with anal cancer in Slovenia, and to assess the physical status (episomal and/or integrated) of HPV genome in HPV16 DNA positive cancers.

Methods: 53 formalin-fixed, paraffin-embedded tissue specimens of histologically confirmed anal squamous cell carcinoma obtained from the same number of Slovenian patients were included and tested for the presence of HPV DNA using GP5+/GP6+ PCR and INNO-LiPA HPV Genotyping Extra test. Genomic variants of HPV16 were determined by sequencing of E6 gene. The physical status of HPV16 genome was determined by HPV16 type-specific E2 and E6 multiplex real-time PCR.

Conclusions: HPVs were detected in 47/53 (87.8%) cases of anal carcinomas: HPV16 was found in 44 samples and HPV6, HPV11 and HPV6/HPV16 in one sample each. Among 25 randomly selected HPV16 isolates from anal cancers, four E6 genomic variants were identified; all clustered to the HPV16 European-Asian genetic lineage. HPV16 genomic variant E6-T350G with presumably higher oncogenic potential was found in 19/25 (76%) isolates. Pure integration of HPV16 genome, characterised by the total absence of E2 gene, was determined in only 1/25 (4%) HPV16 positive anal carcinoma. To conclude, the great majority of anal cancers in Slovenia are etiologically linked with HPV16 variants belonging to the European-Asian genetic lineage. Whether HPV integration plays a role also in the HPV-16 mediated anal carcinogenesis remains elusive.

OBJECTIVES: The increasingly widespread application of improved cytological and colposcopic screening techniques has resulted in the identification of a greater number of intraepithelial lesions. Since dysplasia of the vulva is less common than that of the cervix, the general presumption is that dysplasia identified on a Papanicolaou test most likely represents a cervical lesion; we sought to report 4 cases of usual VIN without cervical or vaginal associated lesions detected by LBC preparations.

METHODS: Between August 2010 and November 2011, women were referred to Hospital das Clinicas of Faculty of Sao Paulo State University in Sao Paulo for gynecological examination for different reasons. Cytological samples were taken and women with abnormal results were submitted to colposcopy and biopsies of the worst visible lesion were also taken.

CONCLUSIONS: One thousand eight hundred and sixty six women were included in this study. We found 7 cases of invasive carcinoma (5 Adenocarcinomas- 1 adenocarcinoma, 1 adenocarcinoma of the cervix, 3 endometrial adenocarcinoma and 2 squamous cells carcinoma of the cervix). From these women, four of them presented VIN alone as a result from an abnormal pap smears. In theory, cervicovaginal smears should not contain vulvar cells. However, August et al[1] described 10 cases of abnormal pap smears and VIN. This fact suggests that there must be undescribed episodes of contamination by cells from the vulva. We believe due to the improvement in the cytological screening techniques we might be able of such kind of vulvar diagnosis.

BACKGROUND: HPV is related to the pathogenesis of various cancers, such as oropharyngeal squamous cell carcinoma, which a high incidence has been reported in Puerto Rico (PR). Despite the burden of oral cancer in PR, little is known about this event in anal carcinogenesis. The aim of this study was to determine the prevalence of oral HPV infection (any type), the genotype distribution and correlates associated with oral HPV infection in men older than 16 years of age attending a sexually transmitted infection (STI) clinic in PR.

METHODS: A cross-sectional study consisting of 205 men was conducted. Participants provided a 30-second oral rinse and gargle with mouthwash. A questionnaire was administered, which included demographic characteristics, behavioral and clinical assessment. Descriptive statistics and bivariate analysis were used to characterize the study sample. Variables that achieved statistical significance in the bivariate analysis (p< 0.05) were assessed in multivariate logistic regression.

RESULTS: The mean age of the study sample was 38.5 ± 14.19 years. The prevalence of oral HPV infection among men was 20% (95%CI=14.75%-26.14%) and of HPV type 16 was 2.4% (95%CI= 0.80%-5.6%). Oral HPV prevalence significantly increased over increasing age (p-trend=0.001). Multivariate analysis showed that oral HPV infection was significantly more common among those with increased number of sexual partners (OR=1.02; 95%CI=1.01-1.03) and men who reported lifetime use of cigarettes (OR=3.00; 95%CI=0.98-9.16). CONCLUSIONS: Interventions should focus in increasing awareness of the long-term consequences of HPV among health care professionals who provide services to this group.
INDICATION OF PLANNED NECK DISSECTION FOR OROPHARYNX SQUAMOUS CARCINOMA BY POSITRON EMISSION TOMOGRAPHY AND HUMAN PAPILLOMAVIRUS INFECTION

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Objectives: Patients with human papillomavirus (HPV)-positive oropharyngeal squamous carcinoma (OSCC) respond well to radiation therapy and have good prognosis. After concurrent chemoradiotherapy (CCRT), we employed planned neck dissection (PND) for patients with N2b lesions to control neck lymph node (LN) metastasis. In the present study, we investigated regional control after CCRT using lymph node samples in patients received PND and whether preoperative positron emission tomography CT (PET-CT) and HPV infection in primary lesions could predict residual lesions in LNs.

Methods: From 2006 to 2010, 56 patients with OSCC participated in this study. Of 56, 13 patients with 19 metastatic lymph node areas were treated with PND after CCRT.

Results: There was no significant difference between SUVmax decrease in PET-CT and residual lesions. However, SUVmax values after CCRT (just before PND) were significantly related to residual LN (p=0.022). Of 19 LN regions, 12 showed pathological CR and 7 showed residual LNs. Patients with HPV positive tended to show high CR rates compared with HPV negative (p=0.03). In the lymph node in patients with HPV positive, metabolic tumor volume (MTV) before CCRT were statistically lower than patients with HPV negative (p=0.046).

Conclusions: Present study suggests that PET-CT and detection of HPV infection are useful for prediction of CCRT, and PND is required with cases who show high MTV value and HPV negative.

P 10-3

HPV RELATED CERVICAL DISEASE AND OROPHARYNGEAL CANCER: A CASE REPORT

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Objectives: Human Papillomavirus (HPV), especially HPV 16, is associated with the development of both cervical and oral cancer. We show the case of a woman affected by HPV-related cervical disease and oropharyngeal squamous cell carcinoma (OPSCC).

Methods: A 41-year-old woman arrived at our Colposcopy Center following an abnormal Pap smear result (ASC-H atypical Squamous Cells cannot exclude HSIL) and a diagnosis of moderate cervical dysplasia obtained by a cervical biopsy. The patient was a mild smoker, had undergone tonsillectomy in childhood and suffered from lupus-like disease.

During the first visit she underwent a colposcopy that showed a cervical abnormal transformation zone grade 2. We programmed a laser conization that was performed in November 2010. Histology reported a moderate/severe dysplasia. The cone resection margins were free.

The patient performed a follow-up by colposcopy and Pap smear every 6 months for the first 2 years. Colposcopy and cytology were always negative. The HPV testing showed an infection by HPV 16.

In October 2012 the patient presented to the Head-Neck ER after episodes of hemoptysis; a suspected HPV related lesion was found at the level of the left tonsillar lobe. A biopsy from the mucosa of the left tonsillar pillar and lobe was performed with a result of squamous cell carcinoma with low grade differentiation. The HPV testing detected a high risk HPV and the immunohistochemical analysis resulted positive for p16 (90%).

The patient underwent a face and neck MRI, which showed a mass of the left tonsillar lobe of 23x22x27 mm, with solid consistency. It appeared in contact with the basis of tongue and with the inner face of the medial pterygoid muscle, without a clear cleavage plane. She was treated by chemotherapy (4 cycles of cisplatin) and brachytherapy (33 sessions) at the dose of 60 grays.

The patient was followed at the Head-Neck Center by monthly visits and she underwent a PET on April 2013 which resulted completely negative.

In conclusion, because of this association found in literature and in our case, we think that women with HPV cervical lesions should have regular surveillance for oropharyngeal cancer, whereas women with OPSCC should be encouraged to have diligent cervical screening.

HPV IN PROGNOSIS AND THERAPY OF OROPHARYNGEAL CANCER

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Introduction: Nowadays, it is generally accepted that acceptance of the oropharyngeal cancer (OPC) has increased in the last 20 years nearly twice. Beside the group of tobacco related cancers, there is a group of tumours (mostly OPC) with etiology related to HPV infection. HPV+ associated oropharyngeal cancers have different molecular and epidemiological profile in comparison to HPV- tumours linked to somatic mutations which reflects in clinical behavior. HPV+ tumours have better prognosis, higher radio- and chemosensitivity.

Methods: The main aim of the grant study of ENT clinics in Prague and Brno and Institute of Hematology and Blood Transfusion in Prague is to determine whether the prognostic advantage of patients with HPV+ tumours is treatment modality dependent. HPV status is assessed in a retrospective study in patients of surgical and non surgical branches. We compare therapy response and survival rate in patients treated with radiotherapy, chemoradiotherapy, biological monoclonal antibody treatment and surgery with regard to HPV status.

Conclusions: In all patients direct (HPV DNA, HPV mRNA of E6 and E 2 genes) and indirect (p53, p16 proteins) markers of an active viral infection will be detected.

Preliminary results (2nd year): So far HPV status of 47 tumour samples of patients with non-surgical treatment and 109 of surgical treated patients were evaluated and compared. As the results are only preliminary, number of non-surgical patients is low. HPV+ patients have better prognosis, higher radio- and chemosensitivity. The complete remission was confirmed in 14/82% of HPV+ versus 13/43% of HPV- patients. 3-year survival rate (disease specific survival-DSS) was significantly higher in HPV+ patients in both surgical and non-surgical branches: 75% and 84% versus 40% and 53%.

Conclusion: Our preliminary results confirmed high rate of HPV positive patients (35% in non surgical an 65% in surgical branch). We didn’t confirm lower average age of HPV + OPC (comparable in both groups in our study). Significantly better prognosis of HPV + patients in both branches. Significantly better therapy response in HPV + OPC (higher chemoradiosensitivity of HPV+). Under these conditions the HPV status could become a tool for a more effective stratification and individualisation of oncoligic treatment. HPV+ patients would likely profit from a less aggressive treatment modalities with elimination of mutilating surgery provided equal survival rate. Supported by grant IGA MZ CR NT12483 and NT14337.
Methods: Using the p16INK4a monoclonal antibody (clone E6H4, Histology V-Kit, ROCHE) for immunohistochemical assay we analyzed oropharyngeal specimens of 31 patients: 16 of these were diagnosed as squamous cell carcinoma, 7 as dysplastic lesions and 8 as benign lesions (negative control). In 7 out of the 16 OSCC patients (mean F.U. 36 months) follow up data were available. To radiotherapy. Further studies with a greater number of cases are necessary however to determine more concrete data.

Conclusions: HPV infection is involved in malignant transformation of IP, and high viral load and integration of HPV have an important role in malignant lesion in association with IP.

HUMAN PAPILLOMAVIRUS LOAD AND PHYSICAL STATUS IN SINONASAL INVERTED PAPILLOMA AND SQUAMOUS CELL CARCINOMA

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Objectives: This study investigated prospectively the role of human papillomavirus (HPV) in paranasal inverted papilloma (IP), IP with sinonasal squamous cell carcinoma (SCC), and SCC.

Methods: HPV presence and viral load and physical status of HPV-16 were examined by polymerase chain reaction-based methods using fresh frozen samples and paraffin-embedded samples obtained from 17 patients with IP (IP group), 5 IP patients with SCC (IP and SCC group), and 16 with SCC (SCC group) and 67 with chronic inflammatory lesions (inflammatory group).

Results: The presence of the HPV genome was detected in 29.4%, 40.0%, 25.0 % and 6.0% of patients in the IP, IP + SCC, SCC, and inflammatory groups, respectively. The IP group showed significantly higher HPV-positive rates than the inflammatory group. All types of HPV detected were high-risk HPV, especially HPV-16. The relative HPV-16 copy numbers varied from 2 to 7953 per 50 ng genomic DNA. Viral load was higher in the IP + SCC groups than in the IP and inflammatory groups. In the IP group, no significant relationship was found between HPV-16 viral load and clinical characteristics, or between physical status and clinical characteristics. Both patients with IP and concomitant SCC showed high viral load and integration.

Conclusions: HPV infection is involved in malignant transformation of IP, and high viral load and integration of HPV have an important role in malignant lesion in association with IP.

HPV ASSOCIATION IN MALIGNANT TRANSFORMATION OF SINONASAL INVERTED PAPILLOMA

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Objectives: We designed the present study to investigate a possible association of human papilloma virus (HPV) with malignant transformation from sinonasal inverted papilloma (SIP) to maxillary squamous cell carcinoma (SCC).

Methods: We reviewed clinical chart of patients with maxillary SCC to select those who had history of SIP of ipsilateral side, and found 4 patients who met the criteria. DNA was extracted from formalin fixed paraffin embedded sections of both maxillary SCC and SIP tissues. HPV DNA was detected by a standard nested polymerase chain reaction, while the expression of p16, a surrogate marker for HPV, was examined immunohistochemically.

Results: Although 1 of 4 (25%) maxillary SCCs was p16-positive, all of SIPs, including a counterpart of the p16-positive maxillary SCC, were p16-negative. Moreover, HPV DNA was never detected in either maxillary SCCs or their corresponding SIPs.

Conclusions: We addressed for the first time HPV status of both SIP and successive SCC from the same individuals. It seems most likely that malignant transformation from SIP to maxillary SCC is independent of HPV.

P16INK4A PROTEIN EXPRESSION IN OROPHARYNGEAL TRACT RELATED TO RADIOTherAPY FOLLOW UP

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Objectives: Head and Neck Squamous Cell Carcinomas (HNSCC) are the 6th most common cancer worldwide. Only recently, a rise in the incidence of oropharynx cancer has been associated with HPV positivity. This shift in the demographics of HNSCC may be related to a decreasing population of patients with tobacco exposure, increasing HPV exposure and better molecular detection of HPV markers. Several reports have shown that HPV-positive Oropharyngeal Squamous Cell Carcinoma (OSCC) has a better clinical outcome than HPV-negative ones (1, 2, 3) following radiotherapy. The aim of this study was to analyze p16INK4a expression in oropharyngeal lesions and to evaluate the follow up of patients receiving radiotherapy.

Methods: Using the p16INK4a monoclonal antibody (clone E6H4, Histology V-Kit, ROCHE) for immunohistochemical assay we analyzed oropharyngeal specimens of 31 patients: 16 of these were diagnosed as squamous cell carcinoma, 7 as dysplastic lesions and 8 as benign lesions (negative control). In 7 out of the 16 OSCC patients (mean F.U. 36 months) follow up data were available.

Conclusions: In our cohort p16INK4a expression was strongly expressed in 5 out of 16 OSCC. In 2 of these (squamous cell carcinoma of the uvula and lingual mucosa) p16INK4a expression was positive, but its distribution pattern corresponded dysplastic areas rather then carcinomatous areas. Two of these 16 cases presented a sporadic p16INK4a immunoreaction in superficial cells, whereas the remaining 9 samples were negative for p16INK4a expression. Regarding the dysplastic lesions, 1 out of 7 were positive for p16INK4a expression. Seven of 8 benign lesions were negative for p16INK4a expression. Follow-up data were available only in 7 cases where strong/sporadic p16INK4a immunoreaction was detected: longer D. F. S. was observed in 2/5 positive cases and in 1/2 cases with sporadic p16INK4a immunoreaction, whereas the other case with sporadic p16INK4a expression was in progression. The remaining two OSCC p16INK4a positive cases were still in treatment.

Despite the small number of patients analyzed, our follow-up data showed that p16INK4a expression in OSCC could be a predictive marker of response to radiotherapy. Further studies with a greater number of cases are necessary however to determine more concrete data.

KERATIN 17 MAY BE A NOVEL PROGNOSTIC MARKER INDEPENDENT OF HPV STATUS FOR HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Objectives: Head and neck squamous cell carcinoma (HNSSC) is the sixth most common malignancy in the world and is a cancer that is associated with a poor survival outcome. Currently, HPV status is the primary prognostic marker for HNSSCs. However, identification of high-risk individuals remains limited and therapeutics targeting HPV-positive HNSSCs have not yet been fully established. Recently, we have shown by mass spectrometry that Keratin 17 (K17) is overexpressed in SCCs of the uterine and cervical mucosa. Subsequent studies have shown that high K17 expression is predictive of poor clinical outcome. Given the similarity between cervical cancer and HNSSC with regard to HPV status, we were interested in determining whether K17 may be a novel prognostic marker for HPV-related HNSSCs.

Methods: Cases of tonsillar SCC (n=13), laryngeal SCC (n=6), tongue SCC (n=8), and normal oropharyngeal mucosa (n=8), were selected from the archival Pathology collections of the BioBank at Stony Brook University. Representative sections were processed from formalin-fixed paraffin-embedded tissue blocks, and immunohistochemistry was performed for K17 and p16INK4a by an indirect immunoperoxidase method. Cases were scored based on intensity of K17 expression (2+), and p16INK4a was scored positive in cases that showed a diffuse nuclear +/- cytoplasmic staining. Additionally, PCR detection of HPV-16/18 was used to confirm the HPV status of each SCC case.

Conclusions: Cytoplasmic K17 staining was highly expressed in 21/25 (84%) of the HNSSC cases. In HNSSCs, staining tended to be localized primarily to the less differentiated cells in the periphery of infiltrative nests; in most cases, K17 staining in adjacent normal squamous mucosa was not observed. PCR results indicated that HPV status was positively correlated with p16INK4a expression. K17 expression, however, did not correlate with either p16INK4a expression or HPV status. High levels of K17 expression (defined as >60%) were significantly associated with decreased patient survival (p<0.05 by Log-rank test). In summary, K17 is highly overexpressed in most cases of HNSSCs and may be a negative prognostic marker for patient survival that is independent of HPV status.

HUMAN PAPILLOMAVIRUS IN LESIONS FROM HEAD AND NECK REGION

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INTRODUCTION: Tobacco smoking and alcohol are the main causes of most Head and Neck Cancers Squamous Cell Carcinoma (HNSCC) however a high proportion of these cancers are occurring in patients who never smoked or drink. Human papillomavirus, etiological agent of cervical cancer has been linked to a subset of HNSSC. Up to 60% of oropharyngeal cancers have been associated with oncogenic HPV types, while other head and neck cancers, including oral cavity tumours, have lower HPV prevalence (about 20%). Similar to all other HPV related cancers HPV-16 is the most common type identified in oral cavity cancers followed by HPV-18.

AIM: Detection of HPV in lesions from Head and Neck region

METHODS: We study a total of 202 biopsies samples, 52 females and 150 males with a mean age of 61 years (range 16-93 years) with lesions in Head and Neck region. Real time PCR (SYBR Green) using SPF primers evaluated HPV presence. HPV positive samples were genotyped using Microarrays (PapilloCheck), Inno-Lipa (innogenetics). As Negative, positive and internal control were used MRC5, SiHa and B-globin, respectively.

RESULTS: HPV were detected in 36% (73/202) of the samples, 57 were males and 16 were females. The most prevalent types detected were HPV16 (15/28) and HPV31.

CONCLUSIONS: HPV were detected in 36% of patients with lesions in different location of Head and Neck region. HPV16 were the most prevalent. HPV detection in HNSSC can be useful regarding the therapeutic approach.
**ABSTRACTS**

**P 11-7**

**ARE CUTANEOUS HPV INFECTED ORAL CAVITY CANCER?**

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**Objectives.** Epidemiological and molecular evidences suggest that HPV infection, particularly by HPV-16, may play a role in the pathogenesis of a subgroup of head and neck squamous-cell carcinomas (HNSCC). However, most of studies on HNSCC are focused on the identification of specific mucosotropic High-Risk (HR) HPVs. Our study aims to identify a broader spectrum of HPV types and in particular cutaneous HPV.

**Methods** Three different patient groups for a total of 150 subjects were analysed: Group A, subjects attending outpatients for dental disorders with normal oral epithelium; Group B, patients with leukoplakia, or erythroleukoplakia, or lichen, or papilloma, that can be considered as potentially malignant oral disorders or pre-neoplasia; and Group C, patients with oropharyngeal tumours. Oral rinses with 0.4% sucrose solution, oral mouth swabs and biopsies from neoplastic lesions were collected from all participants. HPV DNA identification was based on nested PCR with different sets of consensus or degenerate primers followed by direct sequencing. Type identification was performed on the basis of > 90 homology with HPV prototype sequences deposited in Gen Bank database with BLAST Software. The transcriptional activity of viral genomes was evaluated through the E6/E7 viral mRNA expression assessed by RT-PCR with specific primers. In a limited number of samples to determine presence/absence of viral-induced field cancerisation or multifocal infection, normal surrounding tissues were also analysed.

**Results and conclusions** The analyses of samples evidenced that: i) cutaneous HPVs were present in normal oro-opharynx as well as in patients with pre-neoplastic lesions and to a lesser extent in HNSCC. ii) E6/E7 transcripts of cutaneous HPVs were never revealed.

On the other hand HR mucosal HPV DNA and their E6/E7 transcripts were consistently detected mostly in cancer samples, confirming the association of mucosal HPVs with HNSCC. Although the absence of viral transcripts from cutaneous HPV seems to indicate a limited involvement of these HPVs nevertheless it cannot be ruled out their association through a mechanism based on the transient early expression of viral gene altering the normal cellular behaviour.

Data on viral-induced field cancerisation will be presented.

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**P 11-8**

**ORAL AND GENITAL HPV INFECTION OF ASPYMTOMATIC MEN PARTICIPATING FINNISH FAMILY HPV (FFHPV) STUDY**

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**Objectives.** More data are urgently needed to better understand the natural history of male HPV infections. Prevalence, incidence, clearance and persistence of oral and genital HPV infection and, their risk factors, were assessed on men participating FFHPV-Study during 7-year-follow-up (FU). Also the genotype specific concordance among the spouses was determined at baseline.

**Methods.** Asymptomatic men (n=131) were enrolled together with their spouses and sampled by oral scrapings at baseline, and at 2-, 6-, 12-, 24-, 36- and 72-month-FU-visits to accomplish outcomes of oral HPV infections. In addition, genital samples were taken at baseline (semen, urethra) and 72-month-FU-point (urethra, genital). HPV genotyping was performed by using nested PCR and Multimetrix Assay. Covariates of the outcomes were analyzed using generalized equation (GEE) and Poisson regression. At 72-month-FU-visit, clinical examination was performed by a dentist.

**Results.** At baseline, asymptomatic HPV infections were common in both spouses (men oral 18.3%, genital 35.9%; women cervical 18.8%, oral 17.2%). Genotype-specific concordance among the spouses was low ranging from 0% to 9% according to anatomical sites sampled. Female risk behavior correlated with HPV concordance (p=0.030). Sexual habits among the spouses did not show substantial changes during the FU. Changing the partner (OR=15.00, p=0.028) and marital status (p=0.001) increased the risk of incident genital HPV infections in men. Altogether, 17 HPV genotypes were detected in male oral mucosa point prevalence varying from 15% to 31% while incidence time varied from 3.9 to 25.7 months. Of the 74 HPV-positive men, 72% cleared their oral HPV infection. No independent predictors were identified for either occurrence or cleared infections of species 7/9. Genotype-specific oral HPV persistence was detected in 14% (18/129) of men, the mean persistence time ranging from 6.0 to 30.7 months. Smoking increased the risk while previous genital warts protected for oral HR-HPV-persistence (OR, p=0.0001; OR=0.41). At 72-month-FU visit, 50% of the men had mucosal changes, associated only with smoking (p=0.046).

**Conclusions.** HPV DNA carriage in oral mucosa of men was common HPV16 representing the most prevalent genotype. Most of the infections cleared and stable marital relationship protected against the incident infections. Smoking increased the risk of oral HR-HPV persistence supporting its importance as one of the co-factors of HPV infection.

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**P 12-1**

**HIGH-RISK HPV INFECTION IN BILATERAL CONJUNCTIVAL SQUAMOUS CELL CARCINOMA AND UTERINE-CERVIX: A CASE REPORT**

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**Objectives.** We report a case of bilateral ocular surface squamous neoplasia (OSSN) with concomitant High Risk HPV (HR HPV) infection.

**Methods.** We hereby describe the case of a 56-year-old woman, with a persistent right conjunctival nodule apparently secondary to hot oil trauma. Histopathology of the lesion showed a microinvasive conjunctival squamous neoplasia.

After a few months, a papillomatous conjunctival lesion was observed in her left eye, which upon removal was found to be a moderate conjunctival intraepithelial neoplasia (CIN II).

A colposcopy and pap-test were performed in uterine-cervix and resulted negative for cervical intraepithelial neoplasia.

HPV virus detection was performed in both conjunctivae and uterine-cervix.

A Giagen HPV Sign Genotyping Test on a right conjunctival brush yielded a positive result for HPV 39 (HR HPV). A left conjunctiva biopsy obtained from dysplastic tissue was positive HPV 16 and 31 (HR HPV).

**Conclusions.** Interesting features of this case were:

- almost simultaneous dysplastic/neoplastic change in both the eyes;
- presence of different HPV type in the same eye and the two eyes;
- presence in an eye of the same HPV type of the uterine-cervix.

This case highlights the importance of identifying HR HPV related to ocular carcinoma for appropriate prognosis and treatment. HPV infection in the conjunctiva should therefore prompt an investigation of the more common infection sites in the woman, such as the uterine cervix and the lower genital tract.

A HR HPV positive uterine cervix, even in the event of a negative pap test, should be appropriately followed up to prevent cervical neoplasia.
**Differential Humanistic Burden of Genital Warts in Male and Female Patients in Asia**

**Objective:** To investigate the functional and psychosocial effect of genital warts perceived by Asian patients and the individual variations in this effect of genital warts.

**Methods:** A cross-sectional survey was conducted in a STI clinic and two women’s clinics in Singapore. Patients with genital warts completed a questionnaire containing the SF-36, the CECA, and questions assessing socio-demographic and clinical characteristics. Quality of life deficits were determined by comparing the SF-36 scores with local population norms. Variations in SF-36 (norm-based) and CECA scores among patients with different characteristics were examined using multiple linear regressions. Data from male and female patients was analyzed separately.

**Results:** The mean age of male (N=100) and female patients (N=80) who participated in the study was both 31 years; however, higher proportion of male patients was ethnic Chinese, single, affected by recurrent warts, and had tertiary education and history of STDs. Compared with the general population, male patients had similar or better functioning and well-being while female patients had lower levels of productivity, mental health, and general health. Female patients also reported more concerns about their condition than male patients. Among male patients, individuals with a partner were in better quality of life and psychosocial status than those without a partner; patients in recurrent episodes reported lower productivity than those in the first episode. In contrast, the quality-of-life and psychosocial difference was much smaller between female patients with and without a partner and was absent between females in first and recurrent episodes of warts. Alternatively, tertiary education, age of >30 years, and non-smokers were positively associated with better quality of life and/or psychosocial status.

**Conclusions:** Genital warts has differential humanistic effect on men and women in Singapore. Women are more vulnerable to genital warts. They are much concerned about their health and experience reduced productivity. The gender-specific effect identified in this study may have important implications for the management and prevention of genital warts in Asia.

**FREQUENCY OF ANO-GENITAL WARTS AMONG MALES IN THREE CITIES OF NORTHERN ITALY**

**Objectives:** In Italy there are few data on the sexually transmitted HPV-related diseases among males. The potential implementation of an HPV vaccination among young males makes the collection of such data particularly relevant. Considering the inevitable uncertainties of a preliminary study in the male population, we planned to involve selected administrative areas to investigate the incidence of ano-genital warts (AGW) among males. The Universities of Brescia and Padua, and ISS designed, promoted and supported the study.

**Methods:** Three cities (BS, BG and PD) located in northern Italy were involved. In 2012, 29 dermatologists (15 from BS, 12 from PD, 2 from BG) operating both in public and private settings accepted to participate in the study. This network of 29 dermatologists represent a subset of the total number of dermatologists that see patients with AGW, and we cannot estimate which proportion of all the patients diagnosed with AGW in the same areas they cover. For every male patient diagnosed with a first episode of AGW, socio-demographic and behavioral data were collected, as well as information on previous STI and HIV testing. An estimation of the incidence of AGW was calculated using the number of males residing in the municipalities ed as denominator.

**Results:** The 29 dermatologists reported 170 cases of AGW (82 PD; 76 BS; 12 BG). The median study period was 14 months. The median age at diagnosis was 34 years (17-78). Heterosexuals accounted for 79.4% of diagnosed cases, men-having-sex-with-men for 9.4%, bisexuals for 4.1%. A diagnosis of previous STI was reported by 12% of cases. Based on the number of male residents in the three municipalities in the observed age group (75677 in BS, 68330 in PD, 34920 in BG), an overall minimum incidence of 0.96/1000 male residents was observed; specifically, 1.15/1000 in BS, 1.08/1000 in PD, and 0.34/1000 in BG.

**Conclusions:** Our data provide an estimate of the minimum impact of AGW and suggest the need to continue the investigation on the circulation of HPV-related diseases in the male population in view of new prevention campaigns.

**Population-Based Prevalence and Risk Factors for Bacterial Vaginosis and Other Vaginal Pathogens in Young Women in Germany**

**Objectives:** Bacterial vaginosis (BV) is the most common cause of vaginal complaints among women in the reproductive age and associated with adverse pregnancy outcomes and increased risk for acquisition of sexually transmitted infections (STI). We aimed to determine prevalence of BV and other vaginal pathogens among young women in Germany using a newly developed multiplex genotyping assay.

**Methods:** In a pilot study [1] we have demonstrated that self-sampling by cervicovaginal lavage is a well-accepted and reliable method to detect human papillomavirus (HPV) in women. This self-sampling method was now applied to simultaneously test for bacterial vaginosis and a variety of other vaginal pathogens (Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, Neisseria gonorrhoeae, HSV 1 and 2 and various species of Candida, Mycoplasma and Ureaplasma). The vaginal infection panel (VIP) assay recently developed at DKFZ uses multiplex PCR in combination with bead-based hybridization.

We conducted a cross-sectional population-based study among women aged 20-25 years living in Germany and obtained 543 self-collected cervicovaginal lavages. We thereby determined pathogen prevalences and assessed risk factors based on demographic data, sexual behaviour, and previous vaginal symptoms collected by a questionnaire.

**Conclusion:** Prevalence of BV was 20% (95% CI: 17 - 23), which is similar to recent population-based data in the United States. The number of sexual partners, hormonal contraception and consistent condom use in one night stands were independently associated with BV. Our study suggests that self-sampling is a feasible sampling method to determine prevalences of the above-mentioned vaginal pathogens in the general population, and possibly viable to determine individual STI status by convenient sampling at home in combination with multiplexed molecular diagnostic testing.
Chlamydia Trachomatis Genital Infection: Prevalence and Risk Factors

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Objectives: The aim of this study was to evaluate the prevalence of genital chlamydial infection and the associated risk factors in patients in the city of Inhambupe - Bahia to inform the need of screening programs.

Methods: This cross-sectional study was performed by testing chlamydia hybrid capture of endocervical samples in 165 patients undergoing gynecological evaluation in the period between December 11 to December 22, 2010, in a social project called Scientific Flag in the city of Inhambupe – Bahia.

Results: The prevalence of genital chlamydia infection was 7%. The correlation between age and marital status of the patients and genital chlamydia infection was statistically significant. (P = 0.03 and P = 0.049, respectively). Single young patients have a higher risk of developing genital chlamydia infection. Other variables: family income, frequency of gynecological evaluation, history of infertility, previous sexual transmitted disease, use of any contraception method, such as the use of condoms, intrauterine system, hormonal contraceptive, number of sexual partners, age at first intercourse, pregnancies, presence of cervicitis, ectopia, pelvic pain, dyspareunia and vaginal discharge showed no statistically significant correlation.

Conclusion: The screening of adolescents and unmarried women in the municipality of Inhambupe – BA could lead to the eradication of many cases of chlamydia infection by an appropriate antimicrobial therapy, since these patients are at increased risk of acquiring this infection. Furthermore, educational programs to prevent recurrence can be set up in order to reduce the risk of infertility, pelvic inflammatory disease, and ectopic pregnancy.

High-risk HPV Viral Loads and Chlamydia Trachomatis Co-Infections in Women with Low-Grade Cervical Dysplasia

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Objectives: Epidemiological studies demonstrated a causal role of high-risk (HR) HPV in the development of cervical cancer as well as Chlamydia trachomatis (Ct) as an important cofactor in the progression of cervical dysplasia during co-infection. Aim of this study was to evaluate cervical infection of HR-HPV and Ct in our geographical area.

Methods: A longitudinal study was performed to investigate the role of HR-HPV and Ct in cervical cancers. A sample of 231 women, referred to A.O. San Gerardo, Monza, presenting with low-grade cervical dysplasia and followed up for 18 months, was tested using “in house” normalized quantitative TaqMan Real-Time PCR assays specific for HPV 16, 18, 31, 33, 45, 51, 52 genotypes and EVAGREEN Real-Time PCR for Ct.

Conclusions: HR-HPV cervical infection was demonstrated in 57% of patients with a higher prevalence being found for HPV 51, 45 and 16 (26.4%, 22.9% and 18.9%, respectively). Co-infection with multiple HPV genotypes was demonstrated in 44.7%. Ct infection was detected in 16%; the most common serotypes were F, E, K, D, G (36.4%, 27.3%, 15.1%, 12.1% and 9.1%, respectively). HPV and Ct co-infections were found in 15.2%. In a subgroup of 34 patients, who completed follow up visits, HPV incidence, clearance and persistence were further evaluated; new infections were more frequently caused by HPV51, 45 and 16 while viral clearance appeared to occur more frequently for HPV33, followed by HPV51. Persistent infections were prevalently associated with HPV31, 18 and 16. A better understanding of the role of cervical co-infections and HPV viral load in disease progression might improve diagnostic markers in cervical cancer screening.
**LYMPHOGRANULOMA VENereum: CASE REPORT**

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**AIM:** The goal of this case report to highlight the relevance of lymphogranuloma venereum, as well as to establish the need of a precise diagnosis and adequate handling of the disease. Moreover, we compared the clinical outcomes of the studied patient with previous data contained in the literature.

**METHOD:** Case report of a diagnosed patient with lymphogranuloma venereum, admitted in a Public Hospital of South Zone of Sao Paulo. In parallel, we raised and analyzed literature information about the disease using the databases Pubmed, Lilacs, Elsevier and PMC. We selected review articles published between 2009 and 2013 through the selected key words

**CONCLUSION:** Despite being a rare disease, there was an outbreak of cases by L2 genotypes in men who had sex intercourse with other men, which became evident in the Netherlands in 2003, after that the awareness of this disease across Europe. This disease is endemic mainly in parts of Africa, Asia, South America and the Carebean, being rare in industrialized countries. However, many cases have been diagnosed in the Netherlands, and it has been reported in other countries such as Europe, America and Australia since 2004. Currently, the data shows an accumulation of new cases of lymphogranuloma venereum in European countries. Between March 2009 and November 2011, a study conducted to identify cases of this disease in Madrid, showed that the number of cases increased from 10 to 30 and then to 54 during 2009-2011.There are researches that show a resurgence of the disease in both female and male.

As observed in the reported case the disease diagnosis was not confirmed by serology of Chlamydia trachomatis. However, it was noticed that the introduction of empirical treatment with doxycycline had the expected result, getting a better clinic, which goes against the literature

Considering the evolution of this case with literature data we can conclude that I a spite of being a rare disease, the early diagnosis is very important because if this disease is not adequately treated, it can results in serious sequels. The early diagnosis is particularly important because the patient generally perceives the disease symptoms in its secondary stage impairing its diagnosis.

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**RECURRENT AFTER SURGERY DUE TO CERVICAL CANCER, AN EVALUATION OF THE OFFERED FOLLOW-UP PROGRAM**

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**Objective:** During the last 20 years the follow-up program after surgical treatment for cervical cancer has remained unchanged. Surprisingly, little is communicated in relation to the follow-up program even though it has a huge impact on the life of the women and their relatives for five years.

The focus for this study is to evaluate the follow-up program in fulfilling the purpose for early diagnosis of recurrence while reminding and concerning women, who we consider healthy after surgery, 10 times during five years. Already politicians are focusing on the subject due to the socioeconomic consequences, but there is a need for a foundation prior to an adjustment of the follow-up program.

**Methods:** Design: retrospective study of a cohort of women attending follow-up program after surgery due to cervical cancer.

Material: From the patient register at the Department of Gynaecology and Obstetrics, Aarhus University Hospital we identified 567 patients from 1996 to 2011 matching the diagnosis cervical cancer combined with a surgical procedure.

From the Danish Pathology Data Bank the following information is collected:

- Stage of disease - Histology - Surgical procedure - Number of follow-up visit - Results of cytology test after surgery - Date for the last visit without sign of recurrence and/or death - Date for diagnosis of recurrence and/or death

For patients experiencing recurrence and/or death information retrieval from patient files will outline:

- If diagnosis of the recurrence is established at a planned visit or by referral due to symptoms - - Cause of death if necessary

The purpose is to evaluate the follow-up program from 1996 to 2011.

Statistics: Stata 12 will be used to statistical analysis of data. Non-parametrical test will be used.

**Conclusions:** We expect to find a disagreement in the purpose of early detection of recurrence by attending the follow-up program and in the results of the follow-up program and the cytology test. The exploration in how and when the diagnosis of recurrence is made will contribute with new knowledge. We suspect our research to demonstrate recurrence between the planned visits diagnosed due to early referral caused by symptoms. Furthermore we suspect to find that the planned visit makes patients ignore symptoms and delay the time for the diagnosis of recurrence. This would indeed confirm the need for changes in the follow-up program for the benefit of the patients. The study is on going and the results will be presented at the congress.

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**CONSERVATIVE TREATMENT OF CERVICAL ADENOCARCINOMA IN SITU IS SAFE IN WOMEN, WHO WANTS TO PRESERVE THEIR FERTILITY.**

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**Objective:** To evaluate a conservative approach and the prognostic value of endocervical curet-tage (ECC) in patients treated for adenocarcinoma in situ (AIS) of the uterine cervix.

**Materials and Methods:** At our institution all patients with AIS, diagnosed between 1990-2010 and a minimum of 1.5 years of follow-up, were retrospectively identified using a computerised clinical database. During the entire period ECC was routinely performed at the end of the conisa-tion procedure.

**Results:** We found 195 patients with a median age of 32 years and a median follow-up time of 6.4 years. In patients with affected margins, 17% showed recurrent or persistent disease. Among the 64 patients with margin involvement, hysterectomy could be avoided in 58%. Seventeen pa-tients had a second conisation, whereas 27 patients were followed without further treatment. None of these patients treated without hysterectomy had recur- rent intraepithelial neoplasia during the follow up period (median observation time: 5 years).

ECC was performed in 165 patients. In 144 (87.2%) the initial ECC was normal. In 129 no recur-rence was observed during follow-up (89.5%). Positive ECC was observed in 21. Thirteen pa-tients had hysterectomies, 6 hysterectomies were normal. Eight patients, treated conservatively, were without recurrent disease. Two patients with positive ECC did not have a hysterectomy and showed recurrent disease. Among patients with clear margins and negative ECC, 16% had re-current intraepithelial neoplasia (median observation time: 7 years).

**Conclusion:** Adenocarcinoma in situ of the uterine cervix can be treated by conisation without further treatment in fertile women. ECC performed during initial conisation is a prognostic tool for the treatment of AIS. Close follow-up is recommended in women treated conservatively.
THE OVERTREATMENT RISK OF SEE-AND-TREAT STRATEGY IN MANAGEMENT OF ABNORMAL CERVICAL CYTOLOGY

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Objective: To evaluate the feasibility of conization without prior punch biopsy for patient with abnormal cytology.

Study design: A retrospective review was performed of 700 patients who underwent conization at single institution from January 2003 to August 2012. These patients were divided into two groups as a ‘See-and-treat’ group and ‘Three-step’ group depending on whether they underwent punch biopsy or not before conization. The final histologic results of two groups were compared.

Results: Seventy percent of patients (490 of 700) were diagnosed as cervical intraepithelial neoplasia(CIN) II, III, squamous cell carcinoma(SCC) or other malignancy. The overtreatment risk was higher in ‘see-and-treat’ group in patient with atypical squamous cells of undetermined significance/low grade squamous intraepithelial lesion (ASCUS/LSIL) cytology. (64.7% in ‘see-and-treat’ group vs. 36.5% in ‘three-step’ group; p= .001) There was no significant statistical difference in the rate of high grade cervical dysplasia or invasive carcinoma in patient with high grade squamous intraepithelial lesion(HSIL) cytology between groups. (91.8% in ‘See-and-treat’ group vs. 93.5% in ‘Three-step’ group; p=.793)

Conclusion: The patients with HSIL on cytology can be managed by ‘See-and-treat’ strategy with low risk of overtreatment. On the other hands, the ‘Three-step’ management is more appropriate in patients with ASCUS/LSIL cytology.

CO2 LASER TOTAL SUPERFICIAL VULVECTOMY FOR WIDE MULTIFOCAL VULVAR INTRAEPITHELIAL NEOPLASIA GRADE 3: A CASE REPORT.

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Objectives: The ideal treatment of large multifocal vulvar intraepithelial neoplasia grade 3 (VIN 3) in young patients is still debated. At present subjecting young women to classic surgical disfiguring treatments can no longer be justified. The goal is to prevent development of invasive vulvar cancer while preserving normal vulvar anatomy and function. We describe a novel surgical technique that allowed us to treat wide multifocal VIN 3 in an outpatient setting with local anesthesia by the use of CO2 laser.

Methods: A 37-year-old healthy woman with a history of smoking was referred to our institution complaining of vulvar itching. Clinical examination revealed a flat erythematous zone mixed with a thickened, hyperkeratotic, granular white epithelium and some raised warty lesions extending over the larger part of the vulva. Multiple punch biopsy specimens were obtained from the sites of major macroscopic atypia and from the periphery of the area of vulvar involvement with a histological diagnosis of bowenoid VIN 3 in all the specimens. A total superficial vulvectomy was carried out in 2 closer sessions by CO2 laser used in an excisional way. Both procedures were performed in an outpatient setting with the patient under local anesthesia and without suturing stitches or skin flaps. Definitive pathologic analysis confirmed VIN 3 with free margins. No intraoperative and postoperative complications were documented. Functional and anatomic outcomes were optimal. Six-month evaluation revealed an excellent cosmetic result: no scarring appeared on the vulvar surface and no contraction of the introitus was noticed. No relapse occurred after 12 months of follow-up.

Conclusions: VIN 3 is believed to be a precursor of squamous cell vulvar cancer and for this reason it requires active management. With increased incidence of this disease among young women, not only prevention of invasive disease can be considered a primary endpoint, but also preservation of normal anatomy and function. This case suggests that in selected young patients with large VIN 3 involving the entire external genitalia CO2 laser vulvectomy may represent a safe and effective therapeutic option because it provides adequate oncologic management with excellent cosmetic and functional results.

AN INTRACELLULAR SINGLE-CHAIN ANTIBODY FRAGMENT FOR THE THERAPY OF HUMAN PAPILLOMAVIRUS-ASSOCIATED TUMORS

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Objectives. In order to contribute to the development of new molecules for the therapy of Human papillomaviruses-associated lesions, we explored the use of specific antibodies in single-chain format expressed as intracellular antibodies (intrabodies). One intrabody against the E7 oncoprotein of HPV16 (M2SD), expressed in the endoplasmic reticulum of the HPV16-positive SiHa cells, was previously shown able to counteract the activity of the E7 oncoprotein and inhibit proliferation of these cells in vitro (1, 2). Here, we explored the E7 intracellular distribution in relation to the M2SD expression, and used M2SD to revert tumor phenotype of HPV16-positive tumor cells in preclinical animal models.

Methods. SiHa cells were transfected with plasmids expressing the red-fluorescent E7 protein of HPV16 and the M2SD, alone and in combination; both single and double-transfected cells were analyzed by confocal microscopy. To test the antitumor activity of M2SD, two mouse models for HPV tumors, respectively based on the TC-1 and C3 cells, were utilized. The M2SD intrabody was delivered by retroviral system into the tumor cells before cell injection into C57BL/6 mice. Mice were monitored for tumor onset and, after that, tumor burden was evaluated for 5 months.

Conclusions. We showed that confocal immunofluorescence that the M2SD intrabody and the E7 protein colocalize in the endoplasmic reticulum of SiHa cells, suggesting that the M2SD-mediated delocalization of E7 from its usual compartments could account for the intrabody anti-proliferative activity. Furthermore, a marked delay of tumor onset was observed in all the mice injected with the M2SD-expressing tumor cells in both animal models, and a significant percentage of mice remained completely tumor-free. This is the first in vivo demonstration of the antitumor activity of an intrabody directed towards an HPV oncoprotein.

2 (Accardi L et al. BMC Cancer 2011;11:17)
OBJECTIVES: Introduction to Human papillomavirus (HPV) vaccination for female adolescent has been enabled to prevent cervix cancer earlier than before. Unfortunately HPV vaccination in Korea has not yet introduced as a school based vaccine program. Thus it is critical that mothers’ decision to get daughters vaccinated against HPV in Korea. The aim of this study was to identify mothers’ intention to get daughters vaccinated HPV, the factors related to mothers’ acceptance of HPV vaccination for their daughters.

METHODS: A survey design was utilized to collect cross sectional data and data collection was done between July 1 and November 30, 2013. A convenient sample of 1040 mothers having daughter aged 13-18 years in Korea was recruited in this study. Mothers’ intention to get daughters HPV vaccinated, mothers’ factors related demographics and cervix cancer prevention were assessed. Data analysis procedures included χ2 test and Logistic regression analysis using SPSS program (version18.0).

RESULTS: 56.85% mothers answered intention to get their daughters HPV vaccinated. Among the mothers, 46.4% answered heard of HPV, 30.2% answered had talked HPV with daughters, and 19.5% answered had talked Pap test with daughters. In mothers’ cervix cancer prevention behavior, 29.8% mothers did not perform a Pap test. There were significant relationships among mothers’ intention to have daughters get HPV vaccination and HPV test (χ²= 38.91(p<.001), HPV vaccination of mother (χ²= 117.32(p<.001), heard of HPV (χ²= 87.28, (p<.001), had talked HPV with daughters (χ²= 175.21(p<.001), and had Pap test (χ²= 37.29, p<.001). In adjusted Logistic Regression, the determinant of mothers’ intention to get their daughters vaccinated HPV was had talked HPV with daughters (B=0.06, 95 % CI; 0.25-0.13).

CONCLUSIONS: Communication about HPV between mothers and daughters could be necessary to enhance HPV information and recommendation of HPV vaccination for daughters’ cervix cancer prevention. It is needed to more study on factors affecting mothers’ HPV vaccine acceptance related to cervix cancer prevention for their daughters.

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THE HUMAN PAPILLOMAVIRUS: ATTITUDES & BEHAVIOURS TOWARD THE HPV VACCINATION & CONDOM USE, IN RELATION TO CANCER PREVENTION IN MALES.

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OBJECTIVES: The focus of this study is to design and pilot a questionnaire, using the Theory of Planned Behaviour (TPB). By using the Theory of Planned Behaviour the authors examined young male’s attitudes, subjective norms, perceived behavioural control, and intentions in relation to 1) to obtaining the Human Papillomavirus (HPV) vaccine and 2) to use a condom.

METHOD: Three phases of a research programme employing the theory are described: belief elicitation, pilot study and questionnaire development. The focus of this presentation will be solely on the elicitation phase which was conducted to identify the salient beliefs underlying young peoples’ intentions to vaccinate against HPV, and also their intent to use a condom, using semi-structured interviews (n=15) with young males (18 – 28 years). Data analysis were performed utilizing content analysis, which allowed the authors to establish the most frequently cited beliefs which were then selected for use in the main questionnaire.

CONCLUSION: Influential beliefs toward vaccination and condom use were identified. Low levels of awareness of HPV and the HPV vaccine among males were noted throughout the interviews. Nonetheless, the HPV vaccine was mainly perceived positively. However, factors such as cost, having little knowledge of HPV and the HPV vaccine, and potential side-effects impeded participant’s intent to vaccinate. Participants noted how advantages outweighed disadvantages with regard to condom use. Nevertheless, factors such as embarrassment, not being able to communicate with one’s partner, having little awareness of STI transmission, and alcohol consumption were barriers to condom use. Health professionals, friends, and family members accounted for the majority of social influences that contributed to participant’s intentions to obtain the HPV vaccine, and use a condom. Analysis indicated attitudes, subjective norms, and perceived control toward HPV vaccine and condom use could be important predictors of intent.
P 16-4

PARENTS’ THOUGHTS REGARDING THEIR CONSENT TO HPV VACCINATE THEIR YOUNG DAUGHTER IN A SCHOOL-BASED VACCINATION PROGRAMME

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Objectives: The objective of this study was to explore parents’ thoughts regarding their consent to HPV vaccinate their 11–12 year old daughter in a recently started school-based vaccination programme in Sweden. The objective was also to explore their views on HPV-related information.

Methods: Individual interviews with parents (n=27) who had accepted HPV vaccination for their 11–12-year-old daughter were conducted in the spring of 2012. The interviews were recorded, transcribed verbatim and analysed using thematic content analysis.

Conclusions: The parents expressed trust in the recommendations from authorities and a wish to protect their daughter from a severe disease. A school-based vaccination programme was considered convenient by the parents. All this outweighed their concerns about vaccine safety and potential side effects. The school nurse was found to have an important role; the parents wished for a dialogue with her/him to bridge the information gap.

P 16-5

KNOWLEDGE OF HPV INFECTION AND VACCINE ACCEPTABILITY AMONG MALES ATTENDING A SEXUALLY TRANSMITTED INFECTION CENTRE

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Objectives: Data on the knowledge about HPV and acceptability of HPV vaccine among individuals at risk for sexually transmitted infections (STI) are scant. Most studies have been conducted in USA and mainly targeted women, while little is known about males. Among males attending an STI centre in Rome, Italy, we aimed to assess: i) knowledge on HPV infection; ii) acceptability and attitude toward HPV vaccination.

Methods: A self-administered questionnaire including 35 closed questions was distributed to consecutive male attendees of the STI Unit of the San Gallicano Dermatological Institute from April to May 2013. Data on socio-demographic characteristics, sexual identity and STI history (self-reported) were also collected.

Conclusions: 262 males (median age 33 yrs, IQR: 27-40), mostly Caucasian (97.0%), participated. Most of them (69.5%) were men who have sex with men (MSM). Overall, 42.0% had been previously diagnosed with an STI (32.7% of these males were HIV-infected). More than two-thirds of the participants (79.0%) had heard of HPV and most of them (93.2%) knew that HPV is sexually transmitted. Compared to MSM, a significantly higher proportion of heterosexual men (HM) had heard of HPV (74.7% vs. 88.8%). However, the association between HPV and genital warts was only known by 54.1% of the participants, while 77.3% knew that HPV may cause cervical cancer. Only 37.2% of the participants knew of the link between HPV and penile/anal cancer, and most of them were MSM (74.0%).

Around half of the study group (53.6%) had heard of HPV vaccine, with a significantly higher rate among HM (72.0% vs. 45.7%). Overall, 61.0% of the participants were willing to be vaccinated against HPV, with an equally positive attitude among HM and MSM (67.6% vs. 64%). Most of those who were willing to be vaccinated would accept a 3-dose schedule (82.5%) and would pay for the vaccine (67.5%). Efforts should be made to increase the knowledge about HPV among males. The awareness about the role of HPV in the development of genital warts and cancer in men should be especially improved. A positive attitude toward the vaccine was evidenced in most of the surveyed participants.

P 16-6

KNOWLEDGE, ATTITUDE AND PRACTICE ABOUT CANCER OF THE UTERINE CERVIX AMONG WOMEN LIVING IN KINSHASA, THE DEMOCRATIC REPUBLIC OF CONGO

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Objective: The purpose of this study was to estimate knowledge (K), attitude (A) and practice (P) on cervical cancer (CC) and to identify factors associated with KAP scores.

Methods: A cross sectional study was conducted with a sample of 524 women aged 16 to 78 years (median 28 years, interquartile range 22-35) living in Kinshasa. A multistage sampling technique was used to select participants. The women were interviewed at home by trained field workers using a standardized questionnaire. We dichotomized the women’s score on knowledge, attitude and practice as sufficient or insufficient. We then used binary and multiple logistic regression to assess the association between obtaining a sufficient score and a series of socio-demographic factors: age, marital status, education level, occupation, residence and religion.

Conclusions: Obtaining a sufficient score on knowledge was positively associated with higher education (adjusted odds ratio (OR) 3.6; 95% Confidence Interval (CI) 1.6-8.0) and having a formal employment (adjusted OR 2.4; 95% 1.3-4.4); and it was negatively associated with being single (adjusted OR 0.5; 95% 0.3-0.8) and living in the eastern zone of Kinshasa (adjusted OR 0.4; 95% CI 0.2-0.7). The attitude score was only associated with religion. Those women who declared to have a religion other than Catholicism and Protestantism were less likely than the others to get a sufficient attitude score (adjusted OR 0.6; 95% CI 0.4-0.9). Finally, regarding practice, there were negative associations between obtaining a sufficient score on practice and being single (adjusted OR 0.3; 95% CI 0.2-0.5) and living in the eastern zone of the city (adjusted OR 0.4; 95% 0.2-0.7). Although 84% of women had heard about CC, only 9% had ever had a Papanicolaou (Pap) smear test. This study illustrates the low level of knowledge, attitude and practice on CC of women in Kinshasa despite the high incidence of this cancer; it shows that discrepancies exist between K, A and P. Increasing the women’s awareness may be important as the first step in the long chain of conditions to attain a lower incidence and mortality in the DR Congo.
Objective: Singapore started “CervicalScreen Singapore” in 2004; this national cervical cancer screening programme invites women aged 25 to 69 to go for a Pap smear once every three years. In late 2010, Human Papilloma Virus (HPV) vaccination was recommended for girls and women aged 9 to 26 years old. In order to understand the level of knowledge about HPV and its vaccines as well as the barriers towards HPV vaccination, an online survey was conducted. With the insights, a 5-month long educational campaign targeting parents of school-going girls aged 9 to 16 years old was rolled out. A post-campaign analysis was also carried out to examine the effectiveness of the various media channels in reaching out to the target audience.

Methods: The pre-campaign quantitative survey was conducted online, targeting parents with daughters (n=131). The post-campaign analysis was carried out by an external media agency. A review of the HPV vaccination notification numbers (for those aged 9 to 26 years old) to the National Immunisation Registry (NIR) of Singapore from 2010 to 2011 was also undertaken; this information is provided to the NIR on a voluntary basis by health practitioners.

Conclusion: The pre-campaign survey highlighted that across all ethnic groups, the majority of the respondents (Chinese: 57%, Malay: 50%, Indian: 56%) had never heard of HPV. Only 43% correctly identified the relation between HPV and cervical cancer. In addition, 63% were unaware that HPV vaccine can help reduce the chances of cervical cancer; and only 12% had ever searched for information on HPV vaccines. “Lack of information” was stated as the top reason for not vaccinating their daughters. Hence, the campaign adopted a content-rich approach. Overall, it drove 25,174 views to the HPV webpage and 890 views for the educational video. The number of HPV vaccination notification cases (aged 9 to 26 years old) also increased significantly, from 647 in 2010 to 2,978 in 2011.

While the campaign has exhibited a positive impact in driving people to learn about HPV vaccination, specific strategies should be defined to address the translation of knowledge into action, so as to achieve greater vaccine coverage.