This publication contains the abstracts submitted and accepted for the EUROGIN 2011 Congress, held in Lisbon, May 8 - 11, 2011.

For uniformity, all abstracts have been formatted electronically. Occasionally, symbols in electronically submitted abstracts may have been lost or changed in the re-formatting process. Please advise the congress staff of errors that distort the data or change the meaning.

The abstracts have been organized to reflect the scientific program. The first section contains summaries provided by the speakers presenting in the Training Course and the Plenary Sessions, followed by those of the Scientific Sessions, Free Communications and Clinical Sessions.

Abstracts of poster presentations are listed in a separate section. Poster codes correspond to the numbers of the poster boards.

Please refer to the Final Program for a detailed explanation of the coding system.

The codes are also used as a reference in the Index of Authors.

<table>
<thead>
<tr>
<th>Code</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training Courses</td>
<td>TC</td>
</tr>
<tr>
<td>Plenary Sessions</td>
<td>PS</td>
</tr>
<tr>
<td>Main Scientific Sessions</td>
<td>MSS</td>
</tr>
<tr>
<td>Scientific Sessions</td>
<td>SS</td>
</tr>
<tr>
<td>Free Communications</td>
<td>FC</td>
</tr>
<tr>
<td>Clinical Sessions</td>
<td>CS</td>
</tr>
<tr>
<td>Specialized Training Courses</td>
<td>SPC</td>
</tr>
<tr>
<td>Satellite Training Courses</td>
<td>STC</td>
</tr>
<tr>
<td>Eurogin WACC Session</td>
<td>EW</td>
</tr>
<tr>
<td>Posters</td>
<td>P</td>
</tr>
<tr>
<td>Index of Speakers</td>
<td></td>
</tr>
<tr>
<td>Index of Authors</td>
<td></td>
</tr>
</tbody>
</table>

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Human Papillomaviruses infect basal epithelial cells, and can reprogram them to produce an epithelial lesion able to support virus particle production. In such lesions, viral episomes persist in the basal layer at low copy number, but are amplified in the upper epithelial layers as part of the productive cycle. The patterns of viral and cellular gene expression that regulate this are carefully controlled, as are the post-translational modifications and cellular interactions that the viral proteins undergo.

De-regulation of this normal productive infection can occur under some circumstances, such as following infection of the cervical transformation zone, or other susceptible sites. In these instances, expression of viral genes can become elevated, leading to a more dramatic effect on their cellular targets, and in some cases to the accumulation of genetic errors in the host chromosome. Thus the nature of the infected cell and its environment is important for cancer progression along with particular features of the infecting HPV type. These include differences in the activity of viral proteins and their control sequences, as well as the differences in life-cycle strategies and transmission routes used by the different HPV types. Thus HPV16 and HPV18 pose a particular problem at the cervix, with some but not all of the reasons for this being understood.

The development of neoplasia usually requires persistent infection and continuous viral gene expression. In most cases however, lesion regression occurs as a result of a cell-mediated immune response within months or years. The process of lesion-clearance is still poorly understood, but it appears that clearance or latency can result, possibly with reactivation. Current ideas of lesion formation and regression will be discussed, along with emerging views HPV disease at different epithelial sites.

Persistent infections with carcinogenic human papillomavirus types are a necessary cause of cervical cancer. However, HPV infections are very common in the general population and only a small percentage of infections will progress to cancer. In most populations, there is a characteristic peak of HPV infections with a maximum observed a few years after the onset of sexual activity. In contrast to the high prevalence of HPV infections, the risk of cervical precancer and cancer is very low in this age group. In countries with screening programs, precancer prevalence is highest about 10-15 years after the initial peak of HPV infections and cervical cancer prevalence rises about 10 years after the peak of precancers. The described age distributions of the three functional steps in the progression to cervical cancer are important for screening and clinical practice. Due to the high prevalence of HPV infections and the very low risk of disease, population based screening is not effective when starting too early. This is especially important when switching to primary HPV testing is considered. The long progression from precancer to invasive cancer allows to safely extending screening intervals beyond what is the current practice. While the shapes of the age curves are similar in most populations, the age ranges and peak level depend on a multitude of factors, including sexual behavior, immune status, and screening intensity. In some low resource countries, HPV prevalence remains elevated up to an older age which might affect the efficiency of low-cost HPV screening programs in these countries.
CHROMOSOMAL PROFILING OF HIGH-GRADE CIN ARISING FROM PREVALENT VERSUS INCIDENT HIGH-RISK HPV INFECTIONS

Peter J.F. Snijders, Mariska Bierkens, Saskia M. Wilting, Mark A. van de Wiel*, Bauke Ylstra, Chris J.L.M. Meijer, Renske D.M. Steenbergen

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High-grade CIN represents a heterogeneous disease with respect to clinical behaviour. A cross-sectional array CGH study performed on p16\textsuperscript{imm} positive CIN2/3 lesions (n = 46) revealed that they are also heterogeneous with respect to chromosomal profile. Unsupervised hierarchical clustering analysis revealed two clusters, one of which characterized by a relatively low number of alterations. The other cluster contained CIN2/3 lesions with multiple chromosomal aberrations with profiles comparable to those of invasive carcinomas (Wilting et al., Cancer Res 2009). This suggests that these lesions were more advanced in their progression towards malignancy. Subsequently, we determined chromosomal profiles of CIN3 lesions arising from prevalent versus newly acquired (i.e. incident) hrHPV infections in a 5 year period. To that end CIN3 lesions were selected of women with a known 5 year history of hrHPV infection. Eight women had a <5 year preceding hrHPV infection (CIN3<5yrPHI) and 24 had a PHI lasting ≥5 years (CIN3≥5yrPHI). For comparison, 6 CIN3 adjacent to squamous cell carcinomas (CIN3-SCC), their corresponding SCCs, and 6 CIN1 were included. Unsupervised hierarchical clustering revealed two clusters. One cluster, characterized by a low number of chromosomal aberrations, included all CIN1, 75.0% of CIN3<5yrPHI and 37.5% of CIN3≥5yrPHI. Samples in the second cluster, displaying multiple aberrations, included 25.0% of CIN3<5yrPHI, 62.5% CIN3≥5yrPHI, all except one CIN3-SCC and all SCCs. The increase in aberrations in CIN3≥5yrPHI compared to <5yrPHI was highly significant (p=0.002), suggesting that CIN3≥5yrPHI represent more severe lesions. These results indicate that the number of genomic aberrations increase with longer duration of preceding hrHPV infection. The few exceptions of CIN3<5yrPHI with increased chromosomal abnormalities likely represent lesions with preceding HPV infections approaching 5 years in duration.

In conclusion, a longer duration of preceding hrHPV infection is associated with an increased number of chromosomal aberrations in CIN3 and a likely increased short-term risk of cervical cancer.

probability of HPV transmission from one infected partner to another by sex and on the risk factors for transmission.

CYTOLOGY-BASED SCREENING

Amanda Herbert

Guy’s & St Thomas’ NHS Foundation Trust

Cervical cytology achieves high sensitivity in practice by regular repetition of the test and investigation of all levels of abnormality, including persistent minor changes. Higher specificity of the test as a whole is achieved by colposcopy and biopsy, which allows restriction of treatment to high-grade lesions. Evidence of the success of cytological screening is provided by low incidence of invasive cervical cancer (1), except for early screen-detected cancers in young women (2), in well-screened populations and high incidence and mortality in populations without screening.

Widespread implementation of vaccination could eventually reduce the prevalence of cytological abnormalities to the extent that sensitivity as well as positive predictive value might be compromised, leading to its replacement as the primary test by a high-risk human papillomavirus (hrHPV) test. Experience with high-quality cytological screening (3) suggests this would not be justified - unless a test was developed with greater specificity and sensitivity than hybrid capture 2 (HC2), which tests transient infection rather than persistence and may be negative in advanced disease (4).

Conversion of cervical cytology from primary screening to a triage test would also depend on good quality control. As a triage test, high specificity would be required to avoid over-diagnosis of insignificant reactive lesions; so would high sensitivity, to provide confidence in a negative test in the presence of persistent hrHPV. High sensitivity could be achieved by using automated screening for internal quality control in addition to careful ‘manual’ screening, maximizing the benefits of each method. Cytological brooms sample a wider area than punch biopsy and, using liquid-based cytology, allow morphology to be combined with ancillary tests such as hrHPV RNA, p16 and minichromosome maintenance protein 2 (mcm-2). Such combinations, when taken in the context of multidisciplinary communication, could provide a powerful test that enabled treatment or surveillance to be applied respectively to lesions at genuine risk of progression to invasion, or with a known risk of persistence or progression.
IMPLEMENTATION OF GUIDELINES FOR CERVICAL CANCER SCREENING

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The Council of the European Union has recommended since December 2003 the Member States to offer well-organised evidence-based cancer screening through a systematic population-based approach with quality assurance at level appropriate levels. This recommendation includes principles in providing population-based screening for cervical cancer. Based on this recommendation, European guidelines for quality assurance in cervical cancer screening have been published, the 2nd edition published in 2008 (Arbyn et al., eds., 2008; Arbyn et al 2010).

There is not yet much information available on the overall adherence to these recommendations and guidelines. Based on a survey among health authorities of the EU member states (Karsa et al. 2008), approximately 25% of the cervical cytology testing volume took place within the population-based programmes and the majority still within non-population-based activity. About 22% of the potential target population was covered, respectively, by the on-going programmes and 29% of the target population resided in regions with piloting or rollout of the programme on-going.

Another European report (Ronco G and Anttila A, eds, 2009) has collected further information of the programmes, based on the registration practices as included in the recommendation and the guidelines. There were already 15 national or regional screening registries within the EU and the number of registries has been increasing rapidly since earlier surveys. However, many of the registries included just partial information about the programme; indicating that the quality assurance protocols and activities were not yet adhered systematically throughout.

Since these publications, several countries have started a planning process on how to better fulfil the recommendations and guidelines. A new survey need to be performed, taking into account information from the health authorities but also from the available or developing screening registers. A special emphasis should be put on the new member states that share nowadays very high burden of cervical cancers. Efforts are required to follow the adherence and implementation of the guidelines in the European level as a regular activity.

PROPHYLACTIC HPV VACCINE UPDATE

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This presentation summarizes the results and implications of clinical studies published or presented within the last year. Two longer term follow up studies of Cervrix reported virtually 100% sustained efficacy against HPV16/18 persistent infection and CIN up to 6.4 and 7.3 years, respectively. Cervarix was also shown, not surprisingly, to be highly effective against HPV16/18 persistent infection and CIN1+ in Japanese women ages 20-25 over two years. Combined end of study (4 year) efficacy results for the FUTURE I and II trials of Gardasil in young women were published. Protection from vaccine type-related CIN, VIN/ValN and condyloma was very high. Vaccine efficacy in the generally naïve population regardless of type was 30%, 75%, 48%, and 83% for CIN1, VIN1, ValN1, and condyloma, respectively. This efficacy corresponded to rate reductions in naïve women, irrespective of HPV type, of 19.0% for CIN2+, 50.7% for VIN/ValN, 62% for condyloma, 11.3% for Pap abnormalities and 23.0% for cervical definitive therapy. The published Gardasil trial in males, age 16-26, demonstrated an efficacy against external genital lesions caused by vaccine types of 90.4% in the per protocol analysis and 60.2% in the intention to treat analysis. The efficacy of Gardasil against anal infection and AIN in men who sex with men was presented at a U.S. FDA VRBPAC meeting (available on the web). Vaccine efficacy in the per protocol analysis restricted to vaccine types was 77.5% for AIN of any grade and 94.9% for persistent anal infection. Based on these results, the FDA approved the vaccine for prevention of AIN and anal cancer in both men and women.

A number of safety/immunogenicity studies of both vaccines were also published in the last year. Cervarix was shown to be well tolerated and highly immunogenic in adolescent girls (mean age 12 yrs). Co-administration of Cervarix or Gardasil and a diphtheria-tetanus-pertussis-inactivated polivirus vaccine was well tolerated and, compared to the individual vaccines, the combinations were noninferior in immunogenicity against all the antigens in the combined vaccines. Gardasil was tested in trials of HIV infected children (age 7-12 yrs) and HIV infected men (age 22-61). The vaccine was safe in both groups and sero-conversion with >95% for each type. GMTs were marginally lower in the HIV infected children compared to similar aged historical controls. Two alternative dosing schedules of Gardasil were tested. In young women, vaccination at vaccination at 0, 2, and 12 months was noninferior to vaccination at 0, 2, and 6 months. Perhaps less expected, two doses in 9-13 year old girls, at 0 and 6 months, was noninferior to the standard three doses in young women ages 16-26.
Since 2006 two vaccines against human papillomavirus (HPV) infections are available for use in Europe. The process for introducing a new vaccine into the national programme varies in the different countries, but it often occurs in two steps: first a recommendation from a national advisory body is made, and then an official decision is taken by the national health authorities. According to a European survey conducted by the VENICE Project (venice.cineca.org), as of July 2010, the vaccination advisory bodies in 21 of the 29 countries had made a recommendation in favour of HPV vaccination, compared to 12 out of 27 countries in February 2008. Eighteen out of those 21 countries actually implemented the vaccination programme. Few years after HPV vaccines marketing it can be considered an impressive result. Compared with other new vaccines like rotavirus or even pneumococcal vaccine, the decision making process has been relatively quick and straightforward. It reveals the very high interest that both the scientific community and the public health sector expressed for HPV vaccination and its potential impact for cancer control. The main reason provided by those countries who had not introduced HPV vaccination into their national immunisation schedule was financial constraints. The high price of HPV vaccines is actually a new challenge in the field of immunisation. In fact, not introducing HPV vaccination - when a favourable cost-effectiveness profile has been demonstrated – can pose equity issues all around Europe. In such a case, budget constraints should be somehow solved. According to the VENICE survey, 13 out of 18 countries that started the HPV vaccination implemented also a system to monitor vaccine coverage. Coverage with three doses of HPV vaccine varied between 17% and 81% in 2010. Low vaccination levels could be acceptable for those countries that started the programme very recently, but they may be also a signal of low awareness or other issues related to vaccine acceptance. It is of paramount importance to share at European level those good practices that showed to be effective to reach very high coverage levels even few months after the campaign start.

**INTEGRATION OF VACCINATION AND SCREENING:**

**Saraiya M, Lawson H:**

*Centers for Disease Control and Prevention, Atlanta, GA, US.*

**Background:** An integrated cervical cancer prevention program is one that ideally combines primary prevention and early detection and includes the necessary systems to evaluate changes such as how vaccination may impact screening. **Methods:** Through a comprehensive review of the literature, we will describe progress in both developed and less developed countries on how screening and vaccination efforts are integrated, how national screening programs are using modeling data, how surveillance systems are being implemented to help inform about anticipated changes in screening post-vaccination, and how national organizations are convening experts to collect and discuss evidence needed to inform future guideline development.

**Results and Conclusions:** Report will highlight that most countries in less developed settings that consider cervical cancer a health priority are proposing to introduce vaccination and screening using innovative and varied algorithms and strategies. Report will show that integration efforts among countries in developed settings are in the early stages. Countries with infrastructure support and organized screening and vaccination programs have invested resources into increasing surveillance efforts to measure the impact of HPV vaccine on screening, while others are using modeling.
**RISK OF CIN 2, CIN 3, OR CERVICAL CANCER AT COLPOSCOPY**

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Dept. Ob/Gyn, S.C.P.M.G.-Fontana, Fontana, CA 92335

The appropriate plan (i.e. loop electrosurgical excision procedure, conization, repeat colposcopy at 6 months, or Pap and HR-HPV at 12 months) after colposcopically directed biopsy shows normal or CIN 1 depends on the risk of undiagnosed invasive cancer or CIN grade 2 or greater (CIN 2+). The risks of invasive cancer and CIN 2+ depend on the associated abnormal cervical cancer screening tests, the worst impression at colposcopy, whether the endocervical curettage (ECC) shows CIN 2+, and the patient’s age, history of prior cervical neoplasia, and history of immunosuppression. Tables 1-3 are unpublished data from our review of 1,383 women from SPOCCS I and II with cervical cytology of cancer, HSIL, LSIL, or ASCUS with HR-HPV positive [Pretorius RG et al. JLGTD;2011]. Table 1 shows the risk of invasive cancer and CIN 2+ as functions of the associated cervical cytology, Table 2 shows these risks as functions of the worst colposcopic impression, and Table 3 shows the risk of Cancer, CIN 3, and CIN 2 as functions of whether the ECC showed CIN 2+.

**Table 1**

<table>
<thead>
<tr>
<th>Histology</th>
<th>Cytol cancer</th>
<th>Cytol HSIL</th>
<th>Cytol LSIL</th>
<th>Cytol ASCUS/+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cancer</td>
<td>40.0%</td>
<td>5.2%</td>
<td>0.2%</td>
<td>0.0%</td>
<td>2.2%</td>
</tr>
<tr>
<td>CIN 2+</td>
<td>96.0%</td>
<td>73.3%</td>
<td>16.2%</td>
<td>9.7%</td>
<td>28.7%</td>
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**Table 2**

<table>
<thead>
<tr>
<th>Histology</th>
<th>Colpo cancer</th>
<th>Colpo HSIL</th>
<th>Colpo LSIL</th>
<th>Colpo normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cancer</td>
<td>50.0%</td>
<td>5.2%</td>
<td>1.5%</td>
<td>0.4%</td>
<td>2.2%</td>
</tr>
<tr>
<td>CIN 2+</td>
<td>93.3%</td>
<td>72.6%</td>
<td>36.9%</td>
<td>15.2%</td>
<td>28.7%</td>
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**Table 3**

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<th>Endocervical curettage</th>
<th>cancer</th>
<th>CIN 3</th>
<th>CIN 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 2+</td>
<td>15.4%</td>
<td>66.4%</td>
<td>18.3%</td>
</tr>
<tr>
<td>Normal or CIN 1</td>
<td>5.1%</td>
<td>41.6%</td>
<td>57.2%</td>
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Data in Tables 1-3 should be extrapolated to other colposcopy clinics with caution as it comes from an unscreened population which is older (mean age 40.8 years), has a high proportion of cytology of cancer or HSIL (29.7%), a higher proportion of CIN 2+ derived from women with cytology of cancer or HSIL (76.3%), and a much higher risk of cervical cancer (297/100,000 women) than most colposcopy clinic experiences.

Long ago, it was reported that the risk of invasive cancer in a population with abnormal cervical cytology increased from 1% in women age 10-19 years to 15% in women over age 60 years. [Shingleton HM, et al. Obstet Gynecol;1977] A history of persistently positive HR-HPV increases the risk of subsequent CIN 3+ [Nobbenhuis MAE, et al. Lancet;1999], and although most recurrent CIN is found within 1-5 years of treatment, cases of cancer have been found as late as 20 years after therapy. [Kalliala I, et al. BMJ;2005] Lastly, immunosupression (e.g. HIV positive), increases the risk of cervical neoplasia. [Ahdieh L, et al. Am J Epidem;2000]

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**THE ROLE OF HPV TESTING FOR MANAGEMENT OF ABNORMAL PAP SMEARS**

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**Background**

Follow-up recommendations for women with an abnormal Pap smear vary from conservative repeat cytology to immediate referral for colposcopy and biopsy. Given the strong causal relation between infection with oncogenic human Papillomavirus (HPV) types and the development of cervical cancer, detection of nucleic acid sequences of HPV is proposed as a triage method for women with equivocal or low-grade cytological abnormalities and in follow-up after treatment of cervical intraepithelial neoplasia (CIN)

**Methods**

In this presentation, we will give a summary of the main results of recently updated meta-analyses regarding management of women with cervical lesions using HC2 and other HPV assays.

**Results**

HPV triage – in particular with hybrid capture 2 (HC2) – has a significantly higher sensitivity and similar specificity than repeat cytology at cut-off ASCUS+ to detect high grade CIN lesions in women with ASCUS. In women with LSIL, sensitivity of HC2 triage is higher but the specificity of HC2 is significantly lower than repeat cytology to detect CIN2+ lesions. After treatment of CIN lesions, HPV testing has a higher sensitivity and equal or slightly lower specificity than follow-up cytology to detect residual or recurrent cervical lesions.

**Conclusions**

There is enough evidence available to recommend the use of HPV testing in the triage of women with atypical cytology and also in post-treatment surveillance.
NEW EVIDENCES FOR AN ACCURATE FOLLOW-UP

J. Thomas Cox MD
Past-President, American Society for Colposcopy and Cervical Pathology (ASCCP)

Women having an abnormal cervical screening result (Pap test, cotest with the Pap and HPV test, or a positive HPV test as a primary screen) and either having a normal colposcopy or <CIN2 biopsy need follow-up, as do women treated for CIN 2,3 or adenocarcinoma in situ (AIS). How can we best follow women in each of these situations? Several national, regional and international professional societies interested in the prevention of cervical cancer have guidelines on how to manage each of these situations. Additionally, there are numerous new, strong evaluations in the literature that provide direction to creation of new guidelines on post-colposcopy and post-treatment. In this session we will explore the standards in the US (ASCCP 2006 Guidelines) as well as European recommendations in follow-up in these situations. We will also explore the accuracy of each follow-up option and the limitations, both to the limits of what is described in the literature.

IMPACT OF HPV VACCINES ON DISEASE AND MANAGEMENT

Joura E, Medical University Vienna, Vienna, Austria

Objective: To discuss the vaccination of women with HPV related disease

Methods: Review of available data

Conclusions: Women with HPV related disease have definitely been exposed to HPV and are susceptible for persistent infections with further development to disease. This reflects that they are at risk for further disease. Women with genital warts have a substantial risk to develop cervical disease soon after. About one third develops further abnormalities within one year. To date data from the quadrivalent HPV vaccine are available, demonstrating a substantial reduction of subsequent HPV related disease after treatment in women vaccinated before the outbreak or during disease. This includes low and high grade cervical disease. This does not indicate a therapeutic effect. However vaccination of women with HPV-related disease may be discussed on an individual level.
**TC 2-2**

**ANAL NEOPLASIA: EPIDEMIOLOGY, DIAGNOSTICS AND PREVENTION**

Palefsky, J

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**Introduction:** In the United States the incidence of anal cancer has been increasing among both men and women by about 2% per year. Anal cancer is preceded by high-grade anal intraepithelial neoplasia (HGAIN).

**Methods:** Prospective cohort studies, cross-sectional studies, case reports

**Results:** The prevalence and incidence of HGAIN has been studied in only a few populations, primarily HIV-seronegative and HIV-seropositive men who have sex with men (MSM), as well as HIV-seropositive women and women at high risk of HIV infection. Population-based studies show that as many as 25% of HIV-seronegative MSM and 43% of HIV-seropositive MSM have HGAIN. A smaller proportion of HIV-seropositive women have HGAIN (9%). Recent data show that 8% of healthy, HIV-seronegative women with CIN/VIN/VAIN also had HGAIN.

The diagnosis of HGAIN is primarily performed using anal cytology as a screening test, followed by high resolution anoscopy-guided biopsy. Anal cytology has low sensitivity for diagnosis of HGAIN, comparable to that of cervical cytology, although cytology showing a high-grade squamous intraepithelial lesion has a high positive predictive value for HGAIN on biopsy. The role of adjunctive tests such as HPV DNA, HPV RNA-or HPV protein-based tests, or cellular biomarkers such as p16 is still being defined.

Prevention of HGAIN is largely focused on prophylactic HPV vaccines. The quadrivalent HPV vaccine was recently approved by the U.S. Food and Drug Administration for prevention of AIN and anal cancer due to vaccine HPV types in both men and women.

**Conclusions:** HGAIN is common in populations also known to be at highest risk of anal cancer. Anal cancer is more common among women than men in the general population, and women with HPV-related disease at other genital sites such as the cervix, vulva and vagina may be at high risk of HGAIN, and possibly, anal cancer. Among men, MSM are at highest risk, particularly those who are immunosuppressed due to HIV or other causes. This is true of women as well. For the time being, the primary method used to screen high-risk populations is anal cytology, with other testing approaches being actively investigated. Vaccination against HPV is the primary measure to reduce the risk of development of HGAIN due to vaccine types.

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**TC 2-3**

**PENILE ASSOCIATED DISEASES: WHAT THE CLINICIAN SHOULD KNOW?**

Anna R. Giuliano, PhD. H.

*Lee Moffitt Cancer Center, Tampa, FL, US*

HPV infection causes genital warts, pre-neoplastic lesions of the penile epithelium (PIN) as well as penile cancer. Invasive squamous cell carcinoma (SCC) of the penis is rare and accounts for less than 0.5% of all cancers in men worldwide. In the US the annual age-adjusted incidence rate of penile cancer was 0.81 per 100,000 men (1998-2003). Worldwide, areas with a high incidence of cervical cancer also tend to have a high incidence of penile cancer, accounting for up to 10% of all male cancers in some countries. Conversely, a very low incidence has been observed among Jewish populations that commonly practice neonatal circumcision.

Approximately 50% of all penile tumors test positive for HPV DNA. Oncogenic HPV related PIN are believed to be the precursor lesions to warty and basaloid penile carcinomas. Little is known about PIN and its rate of progression to penile cancer. HPV prevalence in penile carcinomas ranges from 14%-100% for any HPV type. A recent systematic review found HPV DNA present in 45.4% of invasive penile tumors after adjusting for PCR primer, histology sub-type, and the year and geographical location of the study. In another review, HPV 16 was the most common type detected (60.2%), followed by HPV 18 (13.3%) and HPV types 6/11 (8.13%). HPV DNA prevalence in penile tumors differs by histological subtype. The most common penile SCC sub-types are keratinizing (49%), mixed warty-basaloid (17%), verrucous (8%), warty (6%), and basaloid (4%). HPV is found in 80-100% of basaloid and warty tumors, but in ~33% of keratinizing and verrucous tumors. The inclusion of different penile SCC sub-types across studies may account for some of the wide range of HPV prevalence reported.

Genital warts are the most common manifestation of genital HPV infection in men. Data from US private health plans estimate that the prevalence of genital warts is highest among men ages 25-29 and decreases with age. In the 1999-2004 National Health and Nutrition Examination Survey, 5.6% of sexually active adults ages 18-59 reported having ever been diagnosed with genital warts (7.2% women and 4.0% men). More than 90% of genital warts are caused by non-oncogenic HPV types 6 and 11.
The incidence of oropharyngeal cancer (OPC) has increased considerably over the past decade in the Western World. This has been linked to the human papilloma virus. HPV-related OPC appears to be a distinct disease entity, which affects younger patients, and which appears to have a much better prognosis than smoking-related head and neck cancer.

In this lecture, we will discuss the evidence for the increase in oropharyngeal cancer and its association with HPV. We will also discuss the significance of HPV on prognostication and how it may affect treatment decisions currently and in the future.

The recommended screening interval varies greatly among countries with widespread cervical screening. In general, many countries with excellent results in cervical cancer prevention have longer screening intervals than some countries with much shorter intervals. In the US, with a tradition of annual Pap test screening, the 2009 American College of Obstetricians and Gynecologists (ACOG) guidelines recommended that women have cervical cytology screening every 2 years between the start of screening at age 21 and age 30. After that, screening was recommended to go to every 3 years for women having three consecutive satisfactory normal Pap tests or a single normal Pap and negative HPV test, presuming no history that puts the patient at higher risk; unfortunately not further defined. Intervals in most European countries are typically much longer, i.e. every 3 to 5 years and there has been discussion that a single negative HPV test likely provides protection at least equal to present Pap interval recommendations for up to 6 to 9 years. One option given in the US ACOG guidelines for cervical screening of women age 30 and over is to screen with a Pap and an HPV test (cotest), and to not screen women negative both tests more often than every 3 years. One large health provider (Kaiser Permanente Northern California) reported in 2009 on the findings from over 800,000 cotests on over half a million women. The overall rate of HPV positive/Pap negative women was 3.99%, which is not burdensome. HPV testing is approximately 92-96% sensitive for CIN 3 and cervical cancer in most studies. Adding the Pap has consistently been shown to increase sensitivity of combined testing to nearly 100%. Hence, cotesting offers very high protection for at least 3 years, and for women not screened at this interval because of the lack of an organized screening system, as in the US, this likely provides protection for much longer for women not coming in for screening at the recommended interval. For countries with high participation in cervical screening in an organized system, prolonging the screening interval to match the level of protection provided by the screening test at the maximum interval is optimal. Prolonging screening intervals, whether based on cytology alone, combined with HPV testing or by HPV testing alone, benefits most women by reducing the risk of detection of transient HPV-induced events not destined to become CIN 3, AIS or cervical cancer. In this session we will look at screening with cervical cytology alone, cotesting, and HPV testing alone in terms of optimal screening intervals for each, taking into consideration whether the screening structure is, or is not, organized.
CURRENT KNOWLEDGE ON SCREENING OF WOMEN LESS THAN 30
Peter Sasieni

Although cytology base screening is very effective in preventing cervical cancer in young women, it is far from ideal. Many women are treated unnecessarily and others develop cervical cancer (particularly stage one) despite being screened. HPV prevalence is so high in young women in many countries that the specificity (for high grade CIN) of HPV testing would be unacceptably low. What then should be done to improve cervical screening in young women?

I will propose a radically different (risk-based) approach in which the results of screening are used as much to determine the next screening interval as to refer women to colposcopy. Using (consensus) HPV testing triaged by cytology and HPV typing combined with adaptive screening intervals of one, two or five years the proposed screening programme has the potential to harness the excellent sensitivity of HPV testing with an acceptable referral rate.

MANAGEMENT OF CYTONEGATIVE / HPV POSITIVE WOMEN
Castle,
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There is now overwhelming evidence that carcinogenic human papillomavirus DNA testing (HPV testing) is more sensitive albeit less specific than cytologic methods of screening for cervical precancerous lesions. As a consequence of using HPV testing in cervical cancer screening, women who test HPV negative will be more reassured against cervical precancer and cancer for the subsequent 10-15 years compared to cytology screening alone, permitting safe extension of the screening interval. However, more women will be labeled screen positive than cytology i.e., women who test HPV positive, cytology negative. Despite being a lower risk sub-population HPV-positive, cytology-positive women, HPV-positive, cytology-negative women will bear a significant proportion of disease over many years. As shown in the NTCC and PROBOSCAM trials, immediate referral of all HPV-positive women to colposcopy increases the early (lead-time) detection of cervical precancer, which leads to reduce incidence of cervical cancer within a few years of follow-up. A negative consequence of immediate referral of all HPV-positive women results in over-referral of women with benign HPV infections and increased detection and treatment of mostly regressive equivocal precancerous lesions (e.g., cervical intraepithelial neoplasia grade 2). Conversely, one-year follow-up of HPV-positive, cytology-negative women, as is recommended in the U.S., emphasizes the identification of women with persistent HPV infection, the necessary cause of cervical cancer. But as shown in the ARTISTIC trial, one-year follow-up of HPV-positive, cytology-negative women is subject to significant losses of follow-up, thereby negating some or all of the sensitivity advantages of HPV testing although HPV-negative women still enjoy the increased safety against cervical precancer and cancer. The tradeoffs, programmatic sensitivity vs. specificity, of the two management strategies among HPV-positive, cytology-negative women will be discussed as well as the use of biomarkers to stratify risk among this important screen-positive sub-group or alternative algorithms for triaging HPV-positive women to immediate colposcopy.
PS 1-5

EXITING WOMEN FROM SCREENING

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Objective: To review the evolution of criteria for exiting women from screening and examine current evidence.

Methods: Screening histories of women age ≥65 who were diagnosed with cervical cancer between 1988 and 1994, as well as 2003 to 2008 were examined utilizing the organization’s databases and the regional Cancer Registry. Conventional Pap smears were used in the period 1988 to 1994 and cotesting with Pap smears and Hybrid Capture 2 HPV tests were introduced during the latter period.

Conclusions: In 1995, prior to writing our first cervical cancer screening Guideline, we examined the screening histories of the 55 women age 65 and older who were diagnosed with invasive cervical cancer while members of the Kaiser Health Plan during the years 1988 to 1994. We made the observation that only 5 of the 55 had 3 documented negative Pap smears between the ages of 55 and 65 and no abnormal Paps during that period. Hence in our first Guideline, issued in 1996, we recommended those conditions as criteria for stopping screening. This was subsequently adopted by other organizations, with some disagreement about the optimal age. With the advent of cotesting we added a single negative cotest as acceptable in place of the three negative Paps. From 2003 through 2008 there were 56 KP members ≥65 years of age diagnosed with cervical cancer, ranging in age from 65 to 101 (median 73). During the same time period there were 1,323,100 woman-years of membership in that age group. (median 3) and 11 women had 3 consecutive negative Paps prior to diagnosis at an interval of 9 to 92 months from the last negative Pap to diagnosis (median 33 months). 2 of 46,401 women with 1 or more negative cotests at age ≥65 were subsequently diagnosed with invasive cancer during 132,639 women-years of followup (1.5/100,000/year). Those 2 women were among the 11 who had 3 consecutive negative Paps at age ≥65. We conclude that 1) Underscreening remains the central driver of the occurrence of cancer at age 65 and above: Most cervical cancers diagnosed at age ≥65 occur in women who have not met our criteria for stopping screening. 2) A few cancers will continue to occur at age ≥65 despite multiple negative tests, as is true in other age groups. 3) We currently have no evidence demonstrating that these cancers would be prevented with continued screening at ages ≥65.

PS 1-6

CERVICAL CANCER SCREENING AS A POST-VACCINATION SURVEILLANCE ACTIVITY

Franco, E
(McGill University, Montreal, Canada)

Two efficacious prophylactic vaccines against infections with human papillomavirus (HPV) types 16 and 18 have become available since 2006. Universal pre-exposure HPV vaccination has the potential to reduce the incidence of cervical cancer by up to 75%. Vaccination is also expected to have an impact on the rate of cervical cytological abnormalities and of diagnostic and treatment procedures required to manage women with such precancerous lesions. The traditional paradigm of Pap cytology screening may not be a suitable preventive strategy in the era of HPV vaccination. Once the cohorts of young women who are being vaccinated reach the age of screening the prevalence of Pap smear-detectable abnormalities will decrease substantially, which will ultimately affect the positive predictive value of cytology and decrease its cost-effectiveness. It is now widely accepted that molecular testing of cervical exfoliated cells for DNA of high oncogenic risk HPVs is a much more sensitive screening tool than cytology to detect high grade cervical lesions and cervical cancer. Cytologic triage of HPV-positive women can reveal the ones that should undergo colposcopic examination and biopsy and will largely obviate the concerns related to false-positives. With the improved sensitivity to detect existing lesions and the more “upstream” focus on cervical carcinogenesis this strategy could be implemented via longer screening intervals than are currently possible with cytology alone, and thus be cost-saving especially after HPV testing is deployed as a screening tool. However, it is in the post-vaccination era when the cohorts of women vaccinated in their teens enter screening age that this approach may prove most valuable by permitting a surveillance system that can serve two roles simultaneously: monitoring duration of vaccine protection (with HPV typing for those who are positive) and screening for cervical cancer. This approach may prove to be cost-effective in high-resource countries by permitting the integration of current immunization practices and cervical cancer control programmes, thus favouring the sharing of resources and surveillance infrastructure. The establishment of vaccination registries that can be linked to administrative databases of cervical screening utilization and tumor registries is an essential requirement for this process to become an effective monitoring system.

EUROGIN 2011 HPV Associated Diseases and Cancer
NEW EVIDENCE FOR VACCINATION OF MALES
Anna R. Giuliano, PhD.
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HPV infection and related diseases are common in men, with the overwhelming majority of disease in men attributable to infection with HPV 6/11 (genital warts) and HPV 16/18 (cancer). Recently a Phase III trial testing the efficacy of a quadrivalent HPV vaccine against HPV infection and disease was completed. The trial included 3463 heterosexual men (HM) and 602 men who have sex with women (MSM) ages 16-26 years enrolled from 71 sites in 18 countries. HPV DNA from exfoliated external genital cell samples and the anal canal (MSM only) were tested for 14 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) at Day 1 and months 7, 12, 18, 24, 30, and 36. Circulating antibodies to HPV 6, 11, 16, 18 were assessed utilizing a Competitive Luminex Immunoassay. Almost all subjects (97.4-99.2%) sero-converted for HPV vaccine types by month 7. At month 36, 88.95, 94.0, 97.9%, and 57% of subjects were still seropositive for HPV 6, 11, 16, and 18 respectively. In per protocol (PPE) analyses qHPV vaccine significantly decreased external genital HPV 6, 11, 16, 18 infection 90.4% (95% CI, 69.2-98.1) as well as persistent infection 85.6% (97.5% CI, 73.4-92.9). In addition, among MSM qHPV vaccine significantly decreased persistent anal canal infection 94.9% (95% CI: 80.4-99.4) and AIN 2/3 74.9% (95% CI: 8.8-95.4). There was no evidence that qHPV vaccine prevents infection and disease caused by 10 additional oncogenic non-vaccine types of HPV. A full analysis for safety indicates that while the proportion of subjects who reported ≥1 adverse event (AE) was relatively high at 60.1% and 53.7% among vaccine vs. placebo recipients, the incidence of ≥1 systemic AEs was low (31.7% and 31.4%), and there were no vaccine-related serious AEs or deaths. The occurrence of AEs did not increase with successive injections or among those entering the study HPV sero-positive.

REAL LIFE IMPACT OF HPV VACCINE AT THE POPULATION LEVEL:
THE AUSTRALIAN EXPERIENCE

Brotherton JML
National HPV Vaccination Program Register and Victorian Cervical Cytology Registry
Victorian Cytology Service
East Melbourne, Australia

Objectives: To describe declines in HPV related disease observed in Australia to date and methods for ongoing monitoring and surveillance.

Methods: Having implemented an extensive HPV vaccination catch up program for all women aged 12-26 years (using the quadrivalent HPV vaccine) between 2007 and 2009, Australia is already observing an impact on the incidence of genital warts and high-grade cervical abnormalities. Genital warts are being monitored using sentinel surveillance of patients presenting to sexual health clinics around the country and monitoring of cervical abnormalities (cytological and histopathological) is conducted through Australia’s state based Pap Test Registers. In Australia, cervical screening commences at age 18, or two years after first sexual intercourse (whichever is later), meaning that there is an immediate overlap between vaccinated and screened women, and hence a unique opportunity to monitor the impact of the vaccine. Surveillance for other HPV related outcomes is commencing, including for type specific genital HPV infection, type specific CIN3 and incident juvenile onset recurrent respiratory papillomatosis. Data linkage between the National HPV Vaccination Program Register and Pap Test Registers will facilitate monitoring of vaccine effectiveness and participation in cervical screening by vaccination status.

Conclusion: Emerging data on the population impact of HPV vaccination in Australia will be presented.
Objectives
While phase 3 trials have shown that vaccination against human papillomavirus (HPV) types 16 and 18 prevents persistent HPV type 16 and 18 infections and most high-risk HPV type positive cervical intraepithelial neoplasia (CIN) grade 2+ lesions, long-term follow-up of the phase III cohorts is needed to demonstrate that HPV16/18 vaccination prevents CIN3 and invasive cervical carcinoma (CIN3+).

Methods
We used data from the Finnish Cancer Registry for passive follow-up of cluster (age-cohort) and individually randomized cohorts of women born in 1984-1989 to assess incidence rates of CIN3+ in HPV16/18 vaccinated Finnish cohort of the bivalent HPV16/18 vaccine PATRICIA trial participants (N=2,409) and a reference cohort (N=15,744) from the same communities. Six months after the phase III trial was closed in 2009 the cohorts were linked with the Finnish Cancer Registry. A pilot study in 2009 showed that the baseline incidence of CIN3+ was 41 per 100 000 women years in the reference cohort. Knowing that CIN3+ incidence rapidly increases when the cohorts age, the baseline incidence yields 80% power to show 70% vaccine efficacy against CIN3+ in just 5 years.

Results and Conclusions
The phase III trial included intensive clinical follow-up and thorough health education and counselling which may have modified subsequent risk of cervical neoplasia in all trial participants. The incidence rates of CIN3+ need to be validated to enable comparison to a cohort not exposed to clinical intervention. For such validation, comparison of the incidence rates in the PATRICIA study participants who received control (hepatitis A) vaccine at the baseline, and received no cross-vaccination at the study end, will be made. Preliminary data from such comparison of the incidence rates at the beginning of passive follow-up of the PATRICIA trial participants and the reference cohort (comprising 50,000 women years) will be reported.

AN EVALUATION OF THE LONG-TERM EFFECTIVENESS, IMMUNOGENICITY, AND SAFETY OF GARDASIL™ IN PREVIOUSLY VACCINATED WOMEN

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Background
The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of a pivotal randomized, placebo-controlled, double-blind, 4-year study to investigate the safety, immunogenicity, and effectiveness of quadrivalent Human Papillomavirus vaccine (qHPV) on the incidence of HPV 16/18-related cervical intraepithelial neoplasia (CIN) 2 or worse in 16-to 23-year old women (Protocol 015).

Methods
Follow-up of subjects will be accomplished in 2 ways: 1) registry-based follow-up for effectiveness data as well as safety data including but not limited to deaths, cancer, and hospitalizations; 2) active follow-up for blood collection for immunogenicity assessments at years 5 and 10 of the LTFU study. Effectiveness and safety analyses will occur approximately 2 years following completion of Protocol 015 and approximately every 2 years thereafter for 10 years. The current report represents the first of these efficacy and safety analyses. Cohort 1 included approximately 2,700 subjects who received qHPV vaccine at the start of Protocol 015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and qHPV vaccine prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN 2 or worse was estimated by calculating the expected incidence of CIN 2/3 or worse in an unvaccinated (placebo) cohort using historical registry data. The primary analysis approach was per-protocol.

Results
There were 1,080 subjects that contributed to the follow-up period out of a total of 2,195 eligible subjects in the per-protocol population in Cohort 1. In these subjects there were no cases of HPV 16/18-related CIN 2 or worse observed. There were also no cases of HPV 6/11/16/18-related CIN, vulvar cancer, and vaginal cancer observed. However, the follow-up time in person-years is still insufficient to make a definitive statement about the effectiveness of the qHPV vaccine for the current time period.

Conclusions
The qHPV vaccine shows a trend of continued protection in women who were vaccinated up to 7 years previously, although there is as yet insufficient data to confirm that protection is maintained. The qHPV vaccine continues to be generally safe and well tolerated up to 7 years following vaccination.
EVALUATION OF THE EFFICACY OF FEWER THAN THREE DOSES OF A BIVALENT HPV 16/18 VACCINE

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Objective: Three-dose regimens for human papillomavirus (HPV) vaccines are expensive and difficult to complete, especially in low-resource environments where the need for cervical cancer prevention is greatest. We evaluated vaccine efficacy (VE) of fewer than three doses of bivalent HPV 16/18 vaccine (Cervarix) to prevent one-year or longer persistence of new HPV16/18 infections.

Methods: Women in our clinical trial in Costa Rica were randomly assigned to receive three doses of HPV16/18 or Control vaccine. Women were followed for incident HPV16 or 18 infection that persisted in visits >10 months apart. Median follow-up was 4.2 years. After excluding women with no follow-up time or who were HPV16 and 18 DNA positive at enrollment, 5967 women received three vaccine doses (2957 HPV; 3010 Control), 802 women received two doses (422;380), and 384 women received one dose (196;188). Reasons for receiving fewer doses and other pre- and post-randomization characteristics were balanced within dose by arm. Attack rates of incident one-year persistent HPV16/18 infection in the control group were unrelated to number of doses received.

Results: VE was 80.9% for three doses (95%CI 71.1% to 87.7%; 25 and 133 events in the HPV and control arms, respectively), 84.1% for two doses (95%CI 50.2% to 96.3%; 3 and 17), and 100% for one dose (95%CI 66.5% to 100%; 0 and 10). No evidence of cross-protection was observed in the women who received fewer than three doses.

Conclusion: The similarity of the VE estimates by dose in this nonrandomized analysis suggests that two, and maybe even one, dose(s) of the HPV16/18 vaccine are as protective as three doses against persistent HPV-16/18 infection in women without evidence of infection at vaccination. Further, the comparability in the attack rate of HPV infection in the control arm by dose suggests that the VE estimates are not biased.

THE RISK HETEROGENEITY OF HIGH-GRADE CIN

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The key steps in the established cervical cancer progression model are HPV infection, followed by progression to precancer, and finally invasion to cancer. However, only a small percentage of HPV infections will progress to cancer, most infections clear spontaneously, and many precancers regress without treatment. The goal of cervical cancer screening is to identify cancer precursors and remove them before invasion occurs. Currently, the widely accepted treatment threshold is a biopsy-confirmed CIN2 or greater (summarized as high grade CIN, HGCIN). However, it has been demonstrated that HGCIN is a biologically heterogeneous group with variable risk of progression to cancer. Moreover, the classification of HGCIN, and especially CIN2, is only poorly reproducible. Recent prospective studies allow estimating the risk of progression for CIN2 and CIN3. Important factors influencing risk of progression include age and HPV genotype. Molecular studies show that there is heterogeneity within CIN3, which might allow developing more specific markers for real precancers. A better ascertainment of real precancers will help to avoid overtreatment of disease that is likely to regress without treatment.
ASSESSING INEQUALITIES OVER THE WORLD (ACCESS, RESOURCES, EDUCATION) AND POTENTIAL SOLUTIONS

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The presentation will focus on redressing inequalities between and within countries with respect to cervical cancer control. It outlines the burden that the disease places on women and on health services, summarizing global statistics, describing regional and intracountry inequities, and the main reasons for the higher incidence and mortality in developing countries. These reasons include lack of priority for women’s health, lack of evidence based national guidelines, poorly organized health systems and infrastructure, lack of awareness, attitudes, misconceptions and believes, lack of resources, and gender related factors that may reduce women’s power of self-determination and affect the provision and receipt of services.

In addressing solutions to the problem, the discussion will address essential elements of successful programs including the issues of access, resources, education, as well as the rationale for selection of the target group for screening, concluding that a multidisciplinary team approach is critical. Further the presentation will discuss the introduction and implementation of accessible, affordable and effective methods for cervical cancer screening focusing on VIA (visual inspection with acetic acid) and cryotherapy. It will also describe current efforts to address the challenges associated with the implementation and affordability of new technologies such as HPV testing for screening and HPV vaccines in developing countries.

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EPIDEMIOLOGIC, GENETIC, BEHAVIOURAL FACTORS AND THE MALE PARTNER INFLUENCE: ARE THEY IMPORTANT?

Franco, E

(McGill University, Montreal, Canada)

Although HPV infection is the central cause of cervical cancer, only a small percentage of women who are infected go on to develop cervical cancer or its precursors. Most HPV infections detected via molecular hybridization techniques are transient, and are no longer detectable within one to two years. Even among women with persistent infection, HPV alone is not a sufficient cause and much work has been devoted to determining why certain HPV-positive women develop cervical cancer while others do not. The multifactorial model of cervical cancer etiology suggests an interplay of various cofactors. Smoking, high parity, long term use of oral contraceptives, co-infections and immunosuppression have been found to increase risk of HPV infection and cervical cancer. Other factors, such as genetic polymorphisms in the Human Leukocyte Antigen (HLA) system, polymorphisms in some oncogenes, nutrition, insulin-like growth factors (IGFs), and viral factors, have also been identified as contributing to the overall cervical cancer risk. Recent epidemiologic studies have also shed much light on the behavioural characteristics that affect sexual transmission. The 40+ mucosotropic genotypes of HPV are among the most common sexually transmitted infectious agents, with lifetime risks around 80%. Probabilities of HPV transmission between heterosexual partners have been measured per partnership and per act, measures that are essential as parameters for transmission dynamic models to be used in informing the cost-effectiveness of HPV-based interventions, such as vaccination and screening.
PS 3-4

SIGNIFICANCE OF MOLECULAR TESTING/MARKERS AND AGE OF EXPOSURE TO HPV

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American Society for Clinical Pathology, Washington, D.C., USA

Virtually all cervical cancer and its immediate precancerous lesions arise from persisting cervical infections by approximately 15 cancer-associated (carcinogenic) human papillomavirus (HPV) genotypes. A new paradigm of cervical carcinogenesis replaces an older model of stepwise progression from low-grade to high-grade morphological changes and can now be summarized as four reliably measured stages:

1) HPV acquisition,
2) HPV persistence (vs. clearance),
3) progression of a persisting infection to cervical precancer, and
4) invasion. Importantly, the natural history of incident HPV infection is age independent: new HPV infections at any age are mostly benign and only become risky when they persist. That is, each new HPV infection, regardless of age, has the same natural history and probability to persist and progress. This is in contrast to HPV infections detected prevalently, which have persisted for an unknown duration prior detection (left censored). Prevalently detected HPV infections are more likely to persist when found at older ages because of the selection bias for longer-enduring infections that persisted for many years prior to detection. A lack of understanding of this difference in the natural history of HPV infections, incident vs. prevalent, has led to confusion regarding the most effective use of HPV vaccines, which has been promulgated by HPV vaccine manufacturers.

PS 3-5

SPECIFIC POPULATIONS AT HIGH RISK OF CERVICAL CANCER:
SHOULD SCREENING AND VACCINATION BE DIFFERENT?

J. Thomas Cox MD
Past-President, American Society for Colposcopy and Cervical Pathology (ASCCP)

Preventing cervical cancer by prevention of infection with the two HPV types causing 70% of these cancers became a reality in 2006 with the approval of the first HPV vaccine for clinical use. Unfortunately uptake of HPV vaccination is dependent, as in many ways is cervical cancer prevention by cervical screening and treatment of precancer, by economics, by location/access, and by social mores. The result is that those populations at highest risk for cervical cancer and at greatest need for HPV vaccination, cervical screening, or both are most often the most likely not to receive either. Considering the limits on resources that drive many of the decisions on what protective efforts can be made available and what will work best in each setting, how can we best protect the most women from getting cervical cancer? The range of possibilities varies from having the resources to both vaccinate and screen, to populations that do not have the resources to even screen with VIA and treat. Screening and management of abnormal cervical cytology and treatment of cervical precancer also has its costs to women and society, much of which could be prevented with primary prevention with widespread HPV vaccination. The various options for preventing cervical cancer in the reality of the varied settings and opportunities that women across the world live in will be explored.
Successful cytology based cervical cancer programmes remain the model for secondary prevention of cervical cancer, even if cytology as a screening test has been contested by alternative screening methods in recent years. National programmes that targeted maximum coverage of older women (as were instituted in countries such as Finland in the previous century) had the most significant impact on cervical cancer prevention. Coverage, coupled with targeting older women was consistently shown to be associated with marked reduction in cervical cancer incidence, compared to time intervals between screens. Targeting high risk women (i.e. those associated with the epidemiological issues such age at first intercourse, number of partners, smokers, users of contraception etc) goes against the natural history of infection with Human Papillomavirus (HPV) infection which is that the expression of clinical disease is not linear – it is related to the type of HPV, host immune response and co-factors, many of which remain to be defined. This talk will argue the lessons learned about secondary prevention of cervical cancer in the 20th century remain pertinent to the 21st century, regardless of screening test used.

**HUMAN PAPILLOMAVIRUSES IN ORAL CARCINOMA AND ORAL POTENTIALLY MALIGNANT DISORDERS – A SYSTEMATIC REVIEW**

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**Objectives:** The primary aim was to calculate pooled risk estimates for the association of HPV with oral squamous cell carcinomas (OSCC) and oral potentially malignant disorders (OPMD) as compared with healthy oral mucosa as controls. The secondary aim was to examine the effect of sampling technique (tissue vs. exfoliated cells) on these risk estimates of HPV infection.

**Methods:** Systematic review was performed using PubMed (January1966-September2010) and EMBASE (January1990-September2010). Eligible studies included randomized controlled, cohort and cross-sectional studies. Pooled data were analysed by calculating odds ratios, using a random effects model. Risk of bias was based on characteristics of study group, appropriateness of the control group and prospective design.

**Results:** Of the 1121 publications identified, 39 cross-sectional studies met the inclusion criteria. Collectively, 1885 cases and 2248 controls of OSCC and 956 cases and 675 controls of OPMD were available for analysis. Significant association was found between pooled HPV-DNA detection and OSCC (OR = 3.98; 95% CI: 2.62-6.02) and even for HPV16 only (OR = 3.86; 95% CI: 2.16-6.86). HPV was also associated with OPMD (OR = 3.87; 95% CI: 2.46-6.02). In a subgroup analysis of OPMD HPV was also associated with oral leukoplakia (OR = 4.03; 95% CI: 2.34-6.92), oral lichen planus (OR = 5.12; 95% CI: 2.40-10.93), and epithelial dysplasia (OR = 5.10; 95% CI: 2.03-12.80).

**Conclusions:** The results suggest a strong association between HPV and OSCC and OPMD.
HPV16 infection causes a subset of oropharyngeal cancers. As such, understanding the epidemiology of oral HPV infection in the general population is important. Further, there is mounting evidence that oral HPV epidemiology may not parallel cervical HPV epidemiology. Oral HPV infection, present in ~5% of healthy individuals, is rare compared to anogenital HPV infections; HPV16 accounts for ~25% of all HPV infections detected in the oral region. Risk factors for oral HPV infection include older age (in contrast to the cervix), sexual behavior (including oral sex), and tobacco use. Little prospective research has been conducted on the natural history of oral HPV infection, including rates of oral HPV persistence, the putative precursor to HPV-associated oropharyngeal cancer. Thus far, it appears that most oral HPV infections clear in a short period of time (<2 years); increased rates of persistence may be associated with older age and current tobacco use. No longitudinal studies have followed persistent oral HPV infection to head and neck cancer (and a precursor to HPV-associated head and neck cancer has not been identified), thus, the field relies on information predominantly from case-control studies. Biologic markers of HPV exposure (such as oral HPV16 infection and serologic antibodies to HPV) increase the odds of oropharyngeal cancer. The only prospective study conducted to date showed a fourteen-fold increased risk of oropharyngeal cancer associated with HPV16 L1 seropositivity in serum samples collected, on average, 9 years before diagnosis of the cancer. Finally, understanding and summarizing oral HPV epidemiology and risk of head and neck cancer is complicated by heterogeneity (and lack of validation) in methods of oral specimen collection, processing, and/or HPV testing.

In 2007, the International Agency for Research on Cancer (IARC) declared human papillomavirus 16 to be a cause of oropharynx cancer. This declaration was based upon a review of the molecular and epidemiological evidence, which satisfied the classic Bradford Hill criteria. Critical associations specific to HPV-caused cancers (e.g. evidence of viral oncogene function in tumors, sexual behavioral associations, and HPV exposure) are observed for oropharynx cancers. Oropharynx cancers caused by HPV arise from the lingual and palatine tonsils, are non-keratinizing squamous cell carcinomas, and present with early tumor stage (including the unknown primary) and advanced nodal stage (often cystic). Oral-sexual behaviors, serological evidence of HPV16 exposure and oral HPV infection all are strongly and consistently associated with oropharynx cancer. Whether tobacco, alcohol or marijuana are co-factors for this disease remains controversial.
The prognosis of HPV-associated head and neck cancers is significantly better than HPV-negative tumors. In fact, HPV is one of the strongest prognostic indicators for these tumors. Localization of HPV to tumor cells is associated with the best diagnostic certainty. The two primary methods used clinically are p16 immunohistochemistry and in situ HPV16 hybridization (HPV16 ISH). p16 is overexpressed in high-risk HPV positive cells as a result of HPV E7-mediated inactivation of the retinoblastoma (Rb) gene product. As such, p16 is a good surrogate of high-risk HPV infection. In general, p16 has a high sensitivity but suboptimal specificity, while the reverse is true for type-specific HPV-16 ISH leading some to advocate for combined testing strategy1. Detection algorithms and clinical performance of these widely used diagnostic assays will be discussed, as well as the relative value of alternative markers.


The incidence of oropharyngeal cancer (OPC) has increased considerably over the past decade in the Western World. This has been linked to the human papilloma virus. HPV-related OPC appears to be a distinct disease entity, which affects younger patients, and which appears to have a much better prognosis than smoking-related head and neck cancer.

In this lecture, We will discuss the significance of HPV on prognostication and how it may affect treatment decisions currently and in the future, and discuss trials in this field.
Persistent infection with human papillomavirus (HPV) is a necessary condition for the development of cervical cancer, and HPV infection is also associated with a proportion of other ano-genital cancers such as cancer of the vulva, vagina, penis as well as anal cancer. In addition, HPV is suggested to play a role in some non-genital cancers such as head and neck cancer (HNC). The term head and neck cancer covers a broad spectrum of anatomical sites with different strength of association to HPV, pointing to a viral etiology in a subset of head and neck cancers. The HPV-associated HNC sites include especially oropharyngeal cancers (notably lingual and palatine tonsils). In previous studies the prevalence of HPV in HNC has shown a great variation (4-80% of oral cancers, 15-85% of tonsillar cancers, and 14-75% of oropharyngeal cancers).

Results from several studies suggest that oral HPV infection is likely to be sexually acquired. The predominating HPV types are HPV 16 and HPV 18 covering up to 90% of the HPV infections. This indicates that the currently available HPV vaccines may have a preventive effect against HNC in the future.

The burden of HNC cancer, trends over time and potential impact of HPV vaccination on HNC will be discussed.

Objectives: Penile cancer is a rare disease in developed countries with age-standardised incidence rates of 0.1 to 1.5 per 100,000 men. In Africa, Asia and South America it counts for 4.4/100,000. Average HPV prevalence in penile SCC is 47.9%. In 43-80% of cases HPV 16 is found. Non HPV-related risk factors for invasive penile cancer are lack of circumcision during childhood, phimosis and cigarette smoking. Circumcision, good personal hygiene and condom use, decrease the risk substantially.

Before proposing prophylactic HPV vaccination, we must calculate the potential impact of the vaccine. We looked for the prevalence of HPV DNA in penile cancers in Belgium.

Methods: Seventy-five paraffin embedded samples were searched for the presence of HPV, by means of RT-PCR. Twenty-one samples were excluded from further analysis because no human nor HPV DNA was found. Sixty-seven per cent (67.4%) of human DNA positive samples (33/49) showed HPV DNA. Five human DNA negative samples contained HPV DNA, resulting in a 70.4% presence of HPV DNA (38/54). HPV DNA types were identified 61 times: HPV 16 was found in 52.5%, followed by HPV 56 (9.8%). HPV type 18 is as prevalent as type 11: 4.9%. Mono-infections were found in 29/38 HPV positive cases (76.3%). Two specimens showed a single infection with low risk HPV type 6 and 11.

Conclusion: In Belgium, 70.4% of the penile tumours are associated with HPV, with HPV 16 being the most common type. Should men be vaccinated in order to prevent penile cancer? Prophylactic vaccines may have a role in preventing a subset of penile cancer. On the other hand, the incidence of penile cancer is rather low (in the developed world). Male vaccination should be seen in an attempt to avoid a broader spectrum of male HPV related disease, e.g. in regions of the world where the incidence of penile cancer is high or in well defined high-risk groups (MSM with anal dysplasia).
MSS 1-2

CURRENT KNOWLEDGE ON EPIDEMIOLOGY AND NATURAL HISTORY

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The spectrum of penile diseases in relation to HPV includes condyloma acuminata, penile intraepithelial lesions (PIN) and cancer. However, we will limit this presentation to include benign conditions, penile HPV infection and genital warts. We will consider the literature on penile HPV infection, which are mainly asymptomatic infections. The current data on incidence, prevalence and risk factors will be summarized. Furthermore, we will present data on condyloma acuminata (genital warts) from e.g. our own study performed in a randomly sampled cohort of Danish men.

MSS 1-4

THE GLOBAL SPECTRUM OF HPV RELATED DISEASES IN MALES

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Both low oncogenic risk (LR) and high oncogenic risk (HR) mucosotropic human papillomaviruses (HPVs) cause a broad spectrum of diseases in men worldwide. HPV 6 & 11 are the predominant cause of anogenital warts, occurring on the penis, anus, and surrounding epithelia. Anogenital warts (condylomata acuminata) are a frequent disease, with an incidence of ~1% in men aged 18-45 years, and cause considerable psychological morbidity and healthcare costs. HPV DNA detection studies show that HPV infection is often widespread in the anogenital region, and frequently sub-clinical. HPV 6/11 much more rarely can cause laryngeal papillomatosis. Carcinoma of the penis is usually seen in older men, and ~50% of cases are caused by HR HPV. Pre-cancer of the penis, also known as Bowenoid papulosis, is the precursor HR HPV disease to invasive penile cancer, and is diagnosed at a younger age-range. Men who sex with men (MSM) frequently present with external and intra-anal HPV disease, including warts, pre-cancer (anal intraepithelial neoplasia, AIN), and invasive cancer. HR HPVs are found in ~90% of invasive anal squamous carcinomas. A high frequency of AIN and invasive anal cancer has been observed in MSM with HIV infection in recent years, and various screening strategies are under investigation. The rare Buschke-Lowenstein tumour is a locally aggressive anogenital verrucous carcinoma associated with both LR & HR HPVs. Oral condylomata acquired through oral-genital contact are commonly seen, and are caused by a spectrum of LR HPVs. There has been recent recognition of the role of HPV 16 and other HR HPVs in oro-pharyngeal cancers, and HPV seems to be an increasingly common cause of these malignancies. The presence of HPV in oro-pharyngeal cancer seems to be associated with a more benign prognosis than HPV negative tumours, and laboratory testing may therefore be of clinical value. The key to HPV disease reduction is through primary prevention, both through safer sexual practices, and widespread implementation of HPV vaccination.
HPV is one of the most common sexually transmitted infections among men. HPVs associated with penile lesions have been divided into low-risk and high-risk HPV types. Manifestations of genitoanal HPV infection in the male as in the female are latent infections, subclinical infections and clinically benign genital warts related to low risk HPVs. Penile cancer as well as the precursor lesions penile intraepithelial neoplasias (PIN) are closely associated with high-risk HPVs. Genital warts harbour in about 90% HPV 6 and HPV 11. About 20-44% of genital warts show coinfections of HPV 6, HPV 11 and high risk HPV types with HPV 16 the most frequently detected type.

In analogy to vulvar cancer two different pathogenic pathways seem to exist for squamous cell cancer of the penis. About 40-50% of penile cancers are related to high-risk HPV types (mainly HPV 16). About half of penile cancers are developing in association with chronic lichen sclerosus and are HPV negative.

Early diagnosis and consequent treatment will contribute to reduce the burden of genital warts and PIN. Diagnosis of genital warts requires exclusion of other sexually transmitted conditions and malignant squamous cell neoplasias and precursor lesions. Every atypical lesion has to be biopsied. Pigmented lesions have to be excised entirely and investigated histologically. Despite some recent hopeful therapeutic developments such as topical green tea derivatives, therapy of genital warts remains a medical problem. Both physician- and self-administered therapies are not fully satisfactory. Several current therapies show high recurrence rates. The increasing incidence and burden of disease clearly support the quadrivalent VLP HPV 6, 11, 16 and 18 vaccine for primary prevention of HPV-associated neoplasia and genital warts in men.

Benefit of vaccination has been already noticed in decreasing numbers of genital wart diagnoses in Australia about 1-2 years after initiating vaccination of young women at a high vaccination rate of about 70%.

To date there have been few published prospective studies of the natural history of HPV infections in men. As such the enrollment visit and the placebo arm of Merck’s Phase III Vaccine Trial Among Men (Protocol 020) provides a rich source of information to fill in critical gaps in knowledge regarding male ano-genital HPV infections. The trial included 3463 heterosexual men (HM) and 602 men who have sex with women (MSM) ages 16-26 years enrolled from 71 sites in 18 countries. HPV DNA from exfoliated external genital cell samples and the anal canal (MSM only) were tested for 14 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) at Day 1 and months 7, 12, 18, 24, 30, and 36. Circulating antibodies to HPV 6, 11, 16, 18 were assessed utilizing a Competitive Lumixex Immunoassay. Overall, at the enrollment visit 21% of HM were positive for HPV at one or more anatomic sites tested. HPV prevalence was 18.7, 13.1%, and 7.9% at the penis, scrotum, and perineal/perianal region respectively. Overall HPV prevalence was higher among MSM compared to HM with the majority of the difference due to a higher prevalence of perineal/perianal and anal canal HPV infection. Overall, 48% of MSM were positive at one or more anatomic sites at enrollment; HPV prevalence was 18.5, 17.1%, 33.0%, and 42.4% at the penis, scrotum, perineal/perianal region, and anal canal respectively. Among both HM and MSM higher numbers of sexual partners was associated with increased risk of HPV detection at enrollment. HPV incidence was estimated among the 2031 men (1730 HM and 301 MSM) randomized to placebo. Mirroring results from the prevalence estimates at enrollment, penile HPV incidence was similar among MSM and HM (8/100 p-y). However, a significantly higher incidence of perineal/perianal HPV was observed among MSM (16/100 p-y) compared to HM (4/100 p-y). The rate of acquiring a new HPV infection was highest at the anal canal for MSM (18.9/100 p-y). Among MSM, HPV 6 was the most commonly acquired new infection whereas HPV 16 was the most commonly acquired infection among HM.
Data from observational studies has long suggested that women with uncircumcised male partners are at increased risk of HPV infection and cervical cancer. Three randomized controlled trials of adult male circumcision conducted in Africa demonstrated significantly reduced risk of HIV. The trial in Rakai, Uganda also examined the impact of circumcision on risk of several sexually transmitted infections in the female partners of trial participants. While circumcision of adult men with HIV did not reduce HIV acquisition in their female partners, among partners of HIV-uninfected men, reduced rates of several sexually transmitted infections were observed, including HPV (1). Specifically, female partners of HIV-uninfected circumcised men had a 30% reduced risk of new high risk (HR) HPV detection after two years of follow-up. A modest increased risk of HR-HPV clearance (12%) was also observed, except for HPV16 which was observed to be 43% less likely to clear in women with a circumcised partner. No impact of circumcision on HPV incidence or clearance was observed in female partners of HIV-infected men. Data from these trials provide conclusive evidence of the protective effect of male circumcision from HPV infection in men and their female partners and support a broad, positive, public health impact of male circumcision. However, while a relative reduction in HPV was observed among the female partners of circumcised men, the absolute prevalence of HR-HPV remained quite high at 2-years (28%), and it is unclear whether the impact would translate to reduce prevalence of neoplasia and cancer.

FACTORS INFLUENCING HPV DYSPLASIA AND ANAL CANCER FOLLOWING HPV INFECTION

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While anal cancer is relatively uncommon in the general population, rates of anal cancer can be substantially elevated in men who have sex with men and in HIV-positive populations. It is now recognized that a large proportion of anal cancers is caused by persistent infections with HPV. Similar to cervical cancer, HPV-related anal cancers seem to be initiated in the anal transformation zone and develop through cancer precursors that can be detected and treated before invasion occurs. There is evidence that HPV16 is more common in anal cancer than in cervical cancer, suggesting that the type spectrum associated with anal cancer is more limited compared to cervix. The epidemiology of anal intraepithelial lesions and anal cancer in the general population and in high-risk populations will be reviewed and data on molecular markers for the progression from HPV infection to anal cancer will be discussed.

DIAGNOSIS OF ANAL INTRAEPITHELIAL NEOPLASIA

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Background: Anal intraepithelial neoplasia (AIN) is the probable precursor of invasive anal squamous cell carcinoma. High-grade AIN (HGAIN) is most often caused by high-risk HPV types 16 and 17 and has the greatest potential to progress to cancer while low-grade AIN (LGAIN) is most often caused by low-risk HPV types 6 and 11 with minimal risk of progression to cancer. Although not definitively proven, the ability to diagnose HGAIN and ablate it at an early stage will hopefully prevent progression to cancer.

Discussion: Most often HGAIN is asymptomatic. Much of the diagnosis of HGAIN has been “borrowed” directly from cervical screening. The hallmark of screening is anal cytology performed with a wetted, non lubricated Dacron swab dry mounted or placed into liquid based cytology medium. The digital rectal exam and visual inspection should augment cytology. Cytology is performed blindly and due to natural mucosal folds can miss swab sampling of dysplastic areas. Standard anoscopy may allow visualization of probable dysplastic lesions and could augment cytology screening. Patients with abnormal cytology, palpable or grossly visible lesions suspicious for dysplasia should have high-resolution anoscopy (HRA) which is essentially colposcopy of the anal canal and margin. HRA utilizes acetic acid and Lugol’s solution to visualize epithelial vascular abnormalities including punctuation and mosaicism as well as mass effect, friability and ulceration. Biopsy of suspicious lesions with histology confirmation of diagnosis is critical. Areas suspicious for but not diagnostic of carcinoma must be re biopsied or excised for definitive diagnosis.

Conclusion: Screening for HGAIN relies of both physical examination and cytology. Patients with abnormalities should have HRA with biopsy and histology confirmation of dysplasia.
WHO SHOULD BE SCREENED? IS SCREENING AN EFFECTIVE TOOL?

Laurent Abramowiz 1, Dalila Benabderrahmane 1, Denis Soudan 2.

When diagnosis is early made anal cancer can be very well handled (95% healing rate for grade 1 but less than 50% for grade 4) (1, 2). In general population, this cancer is not frequent (1 to 3/100 000), but we observe now an increasing incidence (61/100 000 per year) among HIV infected young men who have sex with men.

Natural history of anal dysplasia is not well known. Low grade dysplasia (LGD) doesn’t seem a good target for screening because its evolution on cervix is a spontaneous regression most of the time. Many experts have reported on the high risk evolution for HGD to anal cancer even if HGD spontaneous evolution without treatment has never been described (3). The prevalence for HGD is important among HIV-MSM (52% HGD among 357 men) (4). These pre-cancerous lesions can be treated with imiquimod ointment (5) and/or surgical destruction.

But, when recommendations come from governments, the message is different because of lake of data (6). For specialized physicians (7), the screening is based on cytology, high resolution anoscopy (HRA), biopsy and treatment among population at risk (8). Unfortunately, loss of follow-up is 50 to 80% (9). Another limit is accessibility of HRA which is difficult to learn, time consuming and not specifically refunded (in France).

So, we have to find new strategies or to optimize the screening triad. In France, according to our national recommendations (10), we systematically screen all HIV infected patients in our hospital (11). Among the 1206 patients, 307 (25%) had anal condylomas and 86 (7%) HGD. Six anal cancers were diagnosed. However, among high risk anal cancer people with immunodepression and history of anal cancer or recurrent HGD, we also used HRA for screening. Our proposition is to begin screening with easy and not expensive (for us) cad standard anoscopy and to propose HRA for a minority of very high risk people. This option would increase patient’s follow-up. Others tools are to find to improve efficacy for anal screening of people at risk. For example, Goldstone et al recently demonstrate benefit of HPV genotyping like for cervix (12).

To conclude, all HIV-infected patients with anal intercourse or with history of HPV lesions have to be screened. The triad cytology, HRA and biopsy is probably the best tool, but it seems difficult to apply everywhere and for all targeted population. In France, very specialized physicians perform standard anoscopy for this screening with good results.

MANAGEMENT OF HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA AND ANAL CANCER

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Background: Rates of anal cancer are rising in all men and women, but immunocompromised individuals are at highest risk. High-grade anal-intraepithelial neoplasia (HGAIN) is the probable precursor of invasive anal squamous cell carcinoma (ASCC). While not definitively proven it is hoped that ablation of these precursor lesions will prevent progression to ASCC.

Discussion: HGAIN is a mucosal lesion and methods that destroy or remove the lesions should be adequate treatment. Currently multiple treatments exist but efficacy has not been compared in a randomized, prospective, controlled trial. Excision can often be accomplished without standard surgery and affords definitive histologic diagnosis. Patient applied imiquimod has been used successfully as has topical ablation of limited disease with trichloro-acetic acid. Other modalities include cryotherapy. All of these methods can require multiple treatments and patients must be followed to insure full-thickness destruction of the lesion. Modalities requiring specific clinician skill sets studied have been infra-red photocoagulation, cautery ablation and laser ablation. Extensive disease is most often treated in an operating room and if circumferential can be ablated in a staged procedure. Most often, however, HGAIN can be treated in an office-based setting. Recurrence remains high and patients must continue to be followed. ASCC of the anal margin is often treated with wide local excision with 1 cm margins. ASCC within the anal canal is most often treated with chemotherapy and radiation. Treatment is often curative unless lesions are large or metastatic. Morbidity, especially in immunocompromised individuals, including ulceration, stricture, incontinence, neutropenia and pain is often common. Abdominoperineal resection is reserved for recurrence post chemo/radiation. Microinvasive ASCC has not been defined and as such there is no definitive treatment recommendation for this limited disease. Many clinicians resort to local excision of these lesions with close follow-up rather than chemotherapy and radiation.

Conclusions: Treatment of HGAIN centers around destruction or excision of lesions, while ASCC is treated with wide excision or chemo/radiation therapy. Patients must be followed because recurrence is high.
QUADRIVALENT HPV VACCINE EFFICACY AGAINST HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA IN MEN HAVING SEX WITH MEN

Joel Palefsky, for the Male Quadrivalent HPV Vaccine Efficacy Trial Team

Background
Previous data have demonstrated the efficacy of the quadrivalent HPV vaccine (GARDASIL®) against external genital lesions (perianal/perineal/penile intraepithelial neoplasia and condyloma) in men aged 16-26. In this analysis we examined the efficacy of the vaccine specifically against HPV high-grade anal intraepithelial neoplasia (AIN 2+) and anal cancer in men who have sex with men (MSM).

Methods
Data are from 598 MSM aged 16-26 who were randomized to receive vaccine or placebo at enrollment, month 2, and month 6. Subjects underwent detailed anogenital exams as well as sampling from the penis, scrotum, perineal/perianal and anal canal at enrollment, month 7 and at 6-month intervals afterwards. Efficacy analyses were performed in a per-protocol population (seronegative at day 1 and DNA-negative from day 1 through month 7 to the relevant vaccine HPV type). Median follow-up was 2.5 years (post-dose 3).

Results
Vaccine efficacy against HPV 16/18-related AIN 2+ among MSM was 86.6% (95% CI: 0.0, 99.7). Efficacy against HPV 6/11/16/18-related AIN 2+ was 74.9% (95% CI: 8.8, 95.4). When HPV 6/11/16/18-related AIN cases in which more than one HPV type was found were reassigned based on evidence of preceding infection with a vaccine or non-vaccine high-risk HPV type, vaccine efficacy against HPV 6/11/16/18-related AIN 2+ was 91.7% (95% CI: 44.6, 99.8).

Conclusions
These results demonstrate that the quadrivalent HPV vaccine is efficacious in preventing high grade AIN related to HPV 6/11/16/18 in MSM subjects negative to vaccine HPV types at enrollment.

NEW PROFESSIONAL GUIDELINES ON SCREENING AND VACCINATION: PROVIDER AND PRACTICES.

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Objectives: Describe current provider attitudes and practices in the U.S. related to adherence to cervical cancer screening and management guidelines, recommended and non-recommended uses of HPV testing, HPV vaccination practices, and intent about future screening among a vaccinated cohort. Understand current behaviors of U.S. women about cervical cancer screening.

Methods: Review results of current U.S. provider surveys and behavioral surveys from 2000 to 2010 about HPV and cervical cancer-related knowledge, attitudes and practices.

Results and Conclusions: Review will show that most U.S. physicians use HPV tests to triage abnormal Pap tests. Co-testing is a newer, less common practice and there are many issues related to adoption of extending screening intervals. Many providers continue to use the HPV test in non-recommended ways such as low risk HPV testing, HPV testing prior to HPV vaccination, and to triage high grade abnormal Pap results. Behavioral surveys of women will show an improvement in self-reported screening at later ages. In the U.S., providers have adopted HPV testing into screening and management. Providers are open to screening at age 21, but are resistant to extending screening intervals beyond one year. Current screening behavior will have an impact on future screening practices, especially among a fully vaccinated cohort.
Data from 5 large clinical trials showed that overall hrHPV testing in the baseline screening round detects 22% more CIN3+ lesions and 30% more CIN2+ lesions compared to cytology at the cost of 4-6% lower specificity. Moreover, all the trials show in the subsequent screening round (interval 3 or 5 years) that the women who test HPV negative at baseline in the HPV arm have 50% less CIN3+ in the subsequent round compared to women who test cytology negative at baseline in the cytology arm. Moreover, in the NTCC trial also protection against cervical cancer was seen in women who were HPV negative at baseline.

Data from the POBASCAM showed that hrHPV testing in cervical screening can be implemented cost-effectively in women of 30 years and older.

The lower specificity of the hrHPV test for CIN3+ lesions, which in practice might result in over-referral for colposcopy, can be overcome by triaging hrHPV positive women with cytology at baseline and at 6 or 12 months to keep the costs within acceptable limits.

hrHPV tests have a higher reproducibility than cytology and should be clinically validated and performed in labs with experience in molecular testing and accredited for hrHPV testing. Using hrHPV testing in this context will make cervical screening results reproducible, leads to very low false negative results, and opens the way to extending the screening interval. A short overview will be given of the status of implementing hrHPV testing in cervical cancer screening in Europe.

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**MSS 4-2**

HPV SCREENING: CURRENT PROGRAMS AND IMPLEMENTATION PROCEDURES.

EUROPE: THE ITALIAN PROGRAM.


After the conclusion of the second round of the NTCC study, a number of pilot projects of cervical cancer, based on HPV DNA for primary testing, have started in Italy with financial support from the Ministry of Health. The main purpose is evaluating the practical feasibility and the costs in a routine setting, defining the best organisation, developing quality assurance and monitoring systems (including computerised registration and process indicators) and assessing the effect of HPV testing on screening participation. A project based on cluster-randomised (by birth year) invitation of women aged 35-64 years to HPV-based or cytology-based screening started in 2010 in Turin, Reggio-Emilia and Trento. Some 63,000 women were invited in 2010. Higher compliance to invitation to HPV than to cytology was observed in Turin (ratio 1.21; 95%CI 1.17-1.24). However the increase was smaller in Reggio-Emilia (ratio 1.04; 95%CI 1.01-1.08) and a decrease was observed in Trento (ratio 0.86; 95%CI 0.83-0.89). Another project, without control and targeting women aged 25-64 years was started in Valtellina, parts of the Veneto Region, Ferrara, Florence and an area close to Rome (Guidonia). Some 27,000 women were screened by HPV in 2009-10. Both projects use HPV testing as the only primary test. In both projects HPV-positive women have reflex unmasked cytology. Those with ASCUS+ cytology are directly referred to colposcopy while those with normal cytology are recalled after 1 year for repeating HPV testing and are referred to colposcopy if still positive. The proportion HPV positive women judged to have ASCUS+ cytology ranged from 19% (in Trento) to 54% (in Veneto Region).
**Objectives:** Present updated information on use of HPV DNA testing in the U.S. and the process of updating cervical cancer screening recommendations.

**Methods:** Review and describe current recommendations for use of HPV testing for cervical cancer screening and management in the US, and describe methods and processes for determining the most effective methods of preventing cervical cancer and serious precursor lesions and the adverse events associated with these diagnoses.

**Results and Conclusions:** Currently, more than 60 million cervical cytology screening tests are conducted annually in the US. The screening is performed opportunistically with the majority of tests being done in doctors’ offices and clinics by a variety of health care providers. Although liquid-based cytology is the primary screening method, survey reports show that HPV testing is conducted frequently in association with the Pap test, but not necessarily according to current recommendations. Current guidelines in the US recommend against HPV testing of adolescent women (≤ 20), but include use of a high risk HPV DNA probe for triage of women with ASC-US Pap results, and as an adjunct to the Pap test among women >30 years of age. To date, insufficient data are available to evaluate the use of an HPV DNA probe as a primary screening modality, although primary HPV screening trials from Europe and Canada show considerable promise of change if screening intervals can be widened to make HPV DNA molecular testing cost effective. Among current limitations of such a screening option are the absence of an organized screening program in which women are invited to screening on a regular schedule, and the concern for the women who do not participate in cervical cancer screening despite great efforts to include them, and who remain at great risk of serious disease. Whatever the method of screening, considerable educational efforts for providers and the public are necessary to further reduce the burden of invasive cervical cancer and high grade precursor disease.

**Screening with HPV DNA testing can be considered in a number of contexts:**
1) as triage for borderline Pap smears
2) in combination with cytology for primary screening
3) as a primary screening test followed by immediate treatment with cryotherapy if positive
4) primary screen followed by cytology and referral to colposcopy if both positive
5) post treatment follow up. In developed countries with established screening programmes the quest for both greater sensitivity and specificity of secondary prevention strategies has stimulated many clinical trials of HPV DNA testing and cytology. The outcome of key high quality randomized trials will be presented but the summary is that HPV testing significantly increases sensitivity but lacks specificity and may lead to over treatment. Cytology as a second screen adds to specificity but the search for a molecular test that distinguishes clinically insignificant HPV positivity from true cervical cancer precursors is yet to be developed. In developing countries, HPV DNA testing followed by immediate treatment or treatment soon after testing has been evaluated in randomized trials comparing HPV DNA testing to cytology and Visual inspection with acetic acid in both South Africa and India. HPV DNA testing followed by treatment has been shown to be twice as effective in reducing cervical cancer precursors compared to VIA and to significantly reduced the hazard ratio of cervical cancer compared to no effect of VIA or cytology. Large scale implementation of HPV DNA testing and treatment are yet to be implemented in developing countries as most await the arrival of more affordable tests.
MSS 5-1

THE RESPECTIVE PUBLIC HEALTH IMPACT OF SCREENING AND VACCINATION:
WHAT THE ECONOMIC MODELS TELL US.

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Objective: The goal of this talk is to provide a review and summary of recent cost-effectiveness analyses that have examined how the introduction and widespread use of vaccination will affect screening in countries that have established screening programs.

Methods: Cost-effectiveness studies have been conducted for wide range of settings with different screening programs. While the majority suggest that the addition of vaccination to screening will be cost-effective, this depends on a number of factors, including who is vaccinated. Recent results from trials point to new ways to screen women, including use of HPV testing to screen women more or less frequently than on average. In addition, new evidence suggests that boys and men, as well as older women may benefit from HPV vaccines. This talk will review the latest evidence and highlight areas of controversy that point to the need for new analyses of HPV vaccines in settings with established screening programs.

Conclusions: Issues of whom to vaccinate and how we should use HPV testing in the era of vaccines need to be carefully considered and new studies based on recent data conducted in order to inform how we can and should change screening in the era of HPV vaccines.

MSS 5-2

WHAT ARE THE PUBLIC HEALTH PRIORITIES IN THE DEVELOPED WORLD?

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Objective: Most published economic evaluations of HPV vaccination agree that vaccination of girls before the age of sexual debut is likely to be cost-effective. However, questions remain about the optimal use of current and future preventative HPV-related interventions.

Methods: Some of the key public health issues involving prevention of HPV-related disease which could be informed by modelling include the following:
(i) the cost-effectiveness of vaccinating males with a high burden of HPV disease (such as men who have sex with men or HIV positive men),
(ii) optimal use of screening technologies in a post-vaccination landscape,
(iii) comparative value for money of current bivalent and quadrivalent vaccines, and
(iv) assessing vaccine candidates, including those that target additional HPV types besides types 6, 11, 16 and 18. The types of models to address these issues, as well as their data requirements, will be discussed.

Conclusions: Modelling and health economic evaluation can help to inform key priority setting decisions in the coming years.
**THE CLINICAL BENEFIT OF HPV VACCINATION FOR ADULT WOMEN IN THE NETHERLANDS**

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**Objectives:** The use of HPV vaccines in women has been universally approved from 12 up to 26 years of age, but women older than 16 years in 2010 receive no subsidy for the cost of the vaccine from either government or health-insurance companies in the Netherlands. New insights into the efficacy of HPV vaccines may offer renewed arguments to consider HPV vaccination in adult women.

**Methods:** We calculated the clinical benefit of HPV 16/18 vaccination for Dutch women from the age of 17 up to 26 years in 2010, based on a micro-simulation model for cervical carcinogenesis that considers 14 oncogenic HPV types. The model parameters were estimated from a Dutch screening trial and two surveys on sexual behaviour. The baseline infection risks and the anticipated impact of herd immunity from vaccinating 12-16 year-olds were informed by a type-specific HPV transmission model. We made different assumptions for vaccine efficacy, taking account of cross-protection as reported against CIN2/3 and considering not only the case where the vaccine offers protection to naïve women but also the case where the vaccine offers protection against future type-specific infections irrespective of prior exposure or current infection status.

**Conclusions:** The current HPV vaccination programme is predicted to offer little benefit in terms of herd immunity to women older than 16 years in 2010. The clinical benefit of vaccination above 16 years diminishes with age. If the vaccine is considered efficacious against HPV 16/18 among naïve girls, then vaccination reduces the lifetime risk of cervical cancer from 0.52% to 0.24% for 17 year-olds, and from 0.53% to 0.45% for 25 year-olds. The lifetime risk of CIN2/3 treatment reduces from 7.77% to 3.48% for 17 year-olds, and from 8.06% to 6.12% for 25 year-olds. If cross-protection to non HPV 16/18 types is taken into account, the risk of cervical cancer drops to 0.15% for 17 year-olds and to 0.42% for 25 year-olds. The corresponding CIN2/3 treatment rates are 1.70% for 17 year-olds and 5.16% for 25 year-olds. The effect of vaccination on cancer risk is slightly larger if the vaccine is efficacious against all future type-specific infections. The results are sensitive to the anticipated rates of screening adherence, as screening non-attenders remain at increased cancer risk even when vaccinated.

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**POTENTIAL IMPACT OF HPV VACCINATION ON HEALTH INEQUALITIES**

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**Objective:** Rates of HPV infection and disease remain disproportionately high among subpopulations of men and women. The aim of the study is to examine the potential impact of 1) HPV vaccination on health inequalities, and 2) differential vaccine coverage by risk group on overall population-level effectiveness.

**Methods:** An individual-based dynamic model of sequential partnership formation and dissolution, and HPV transmission (18 HPV-types) in a population stratified by age, gender, 4 sexual activity levels (low=L0, high=L3) and HPV type-specific infection status was developed. The distribution of level of sexual activity within our population is L0=27%, L1=53%, L2=19% and L3=1%. Strategies investigated included vaccination of 1) girls only and 2) girls+boys. For each strategy, we varied coverage by level of sexual activity. Population-level vaccine effectiveness is measured by the percentage reduction in HPV-16/18 prevalence in females after 70 years post vaccination compared to no vaccination.

**Conclusions:** Under base case assumptions (age at vaccination=12yrs, per-act vaccine efficacy=100%, average duration of protection=20yrs, overall coverage=70%), vaccine effectiveness of girl only vaccination is 80%, 87%, 58%, 41% for L0, L1, L2 and L3 levels of sexual activity, respectively. Furthermore, under our base case scenario, girl only vaccination assuming uniform coverage across levels of sexual activity results in a population-level vaccine effectiveness of 74% compared to between 45-64% when coverage decreases with higher levels of sexual activity. The predicted inequalities produced by HPV vaccination and the impact of disparities in coverage on vaccine population-level effectiveness generally decreased with increasing population-level coverage due to increased herd-immunity effects. In conclusion, even if vaccination coverage is equal between subgroups of girls, inequalities in HPV prevalence may increase following HPV vaccination. This is because vaccination may produce lower effectiveness within subpopulations that have characteristics which facilitate a more efficient spread of STIs (lower herd immunity).
EUROPEAN HPV VACCINATION PROGRAMMES: ELIGIBILITY CRITERIA AND IMPACT

Hans Berkhof and Hans Bogaards

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2 Centre for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Objectives:
European HPV vaccination programmes show considerable variability in eligibility criteria although full reimbursement of the vaccine is usually restricted to teenage girls. Important reasons for not including boys and young women are that the efficacy of the vaccine is uncertain in sexually active women and that cost-effectiveness calculations have shown unfavourable results. However, recent decreases in the HPV vaccine price have provided an argument to reconsider the eligibility criteria of running programmes and to carefully assess the criteria when installing a new programme. We reviewed vaccination programmes in European countries and studied the impact of HPV vaccination as a function of age and gender on prevalence of high-risk HPV and cervical disease.

Methods:
The impact of HPV vaccination on the cervical cancer incidence was estimated from a 14 type micro-simulation cohort model. The age-specific risks of HPV type infections were obtained from a transmission model.

Conclusions:
Under the assumption that the HPV vaccine is only protective in type-naïve women, vaccination is beneficial if given at least a few years before entering the screening programme. However, vaccinating girls/young women older than 16 years is usually only cost-effective at a vaccine dose price that is substantially lower than the pharmacy price.
In the setting of imperfect vaccine uptake among girls, vaccinating boys is likely to be less effective in reducing the prevalence of high-risk HPV in the population than further enhancing the vaccine uptake among girls.

ABSTRACTS

PRIORITIES IN THE DEVELOPING WORLD: SCREENING VS. VACCINATION

Kulasingam, SL

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Objective: The goal of this talk is to provide a review and summary of recent cost-effectiveness analyses that have studied the impact of vaccination and/or screening on cervical cancer incidence and mortality in resource limited settings.

Methods: Cost-effectiveness studies have been conducted for wide range of settings that do not currently have screening programs in place. While vaccination can be potentially cost-saving in such settings (compared to treating women with cancer), there are a number of important considerations. This talk will review the latest evidence and highlight areas that need to be addressed in order to determine how best to implement vaccination and/or screening in such settings.

Conclusions: HPV vaccine-based programs have the potential to dramatically reduce cancer incidence and mortality in resource poor settings. However, how best to optimize vaccination-based programs and whether and how to combine these with screening and with what type of tests remains to be determined.
ONGOING MODELS AND CRITICAL ISSUES

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1. Laval University, Faculty of Medicine, Canada
2. Imperial College, Department of Infectious Diseases, UK

Objectives: The structure of HPV models has evolved to address questions of increasing difficulty. At first, static models provided timely effectiveness estimates for the vaccination of girls. But for questions about catch-up programs or boy vaccination, models needed to account for herd immunity and hence transmission and mixing were included into dynamic structures. Furthermore, other model assumptions proved to influence significantly vaccine effectiveness results, such as the duration of partnership, the grouping of HPV types or the time function used to represent waning of vaccine protection, and these parameters were also added to the structure. Today, ongoing HPV models will need to integrate even more parameters in order to study current policy questions like, for instance, disparities in population sub groups (e.g. MSM), other HPV-related diseases (e.g. head and neck), or compare the new multivalent vaccines or screening tests. The objective is to present the main structures used to model HPV vaccination with their respective advantages and limitations, and discuss critical issues to address current policy questions.

Methods: The review consists of HPV modeling studies published in the literature from 2002 to 2010. The various model structures were categorized based on level of complexity and research questions. We analyzed the advantages and limitations of each model category.

Conclusions: HPV models have been able to address questions of increasing complexity. However, because the number of parameters and the variety of structures have grown faster than the body of epidemiological evidence, parameter and structural uncertainty has progressively become an issue. Current policy questions will require HPV models to incorporate even more parameters; more heterogeneity in the population, more disease states, more individual genotypes and detailed non-Markov screening algorithms. Under these circumstances, it is important to control the level of uncertainty and identify the parameters that influence the most model predictions. In particular, critical issues are to better understand natural immunity and re-infection, infectiousness and transmission, vaccine efficacy against potential transient infections, the relative progression and regression rates of the various genotypes as well as possible interactions between them.

HOW TO MANAGE SCREEN CYTO NEGATIVE HPV POSITIVE WOMEN

Guglielmo Ronco.
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HPV testing has lower positive predictive value (PPV) than cytology. Therefore referring all HPV positive women for colposcopy causes many unneeded colposcopies. The most studied approach is testing HPV positive women for cytology and referring immediately to colposcopy those who show cytological abnormalities. The remaining are re-tested after 6-18 months and referred to colposcopy only if HPV infection persists. This approach, denoted as “cytological triage” is based on knowledge that only persistent infections are relevant for carcinogenesis. From age 35 years, the reduction of CIN3 in HPV-screened vs. cytology-screened women at round 2 was similar in RCTs that applied cytological triage and in those that referred all HPV positive women to colposcopy, suggesting that protection against cancer is similar. Instead, the PPV of HPV testing with cytological triage is similar to that of cytology while direct referral it is markedly lower. Infection by HPV16 or HPV18 entails higher risk of high-grade CIN than infection with other HPV types. Therefore it has been suggested that these women are immediately referred to colposcopy independently of cytology. One drawback of methods based on assessing infection persistence is loss to follow-up. Other biomarkers as viral load, E6/E7 mRNA and p16-INK4A overexpression are under study. One study nested in a RCT showed that triaging HPV positive women by a single p16-INK4A test has 50% higher sensitivity than cytology while causing a similar number of colposcopies. Recent data also show that HPV positive but p16-INK4A negative women aged 35+ years have very low probability of new CIN3 in the next few years.
**CLINICAL AND ANALYTICAL PERFORMANCE OF THE REALTIME HIGH RISK HPV TEST IN POPULATION-BASED CERVICAL SCREENING SETTINGS**

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1 Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia
2 National Institute of Public Health of Slovenia, Ljubljana, Slovenia
3 Clinics of Obstetrics and Gynecology, Hannover Medical School, Hannover, Germany
4 Department of Obstetrics and Gynecology, University Medical Centre, Ljubljana, Slovenia

**Objectives:** Clinical and analytical performance of the Abbott RealTime High Risk HPV test (RealTime) and Qiagen Hybrid Capture 2 HPV DNA Test (hc2) were compared on 4,432 and 4,479 samples, respectively, originating from a prospectively enrolled Slovenian primary cervical cancer screening cohort.

**Methods and results:** In women above 30 years (N=3,128), clinical sensitivity for the detection of CIN2+ lesions (38 cases) and clinical specificity for lesions less than CIN2 (3,091 controls) of the RealTime were 100% and 93.3%, respectively, and corresponding values of hc2 were 97.4% and 91.8%, respectively. A noninferiority score test showed that clinical specificity (P<0.0001) and clinical sensitivity (P=0.011) of RealTime were noninferior to that of the hc2, at recommended thresholds of 98% and 90%. In the total screening cohort (women 20-64 years; N = 4,432; 57 cases; 4,375 controls) clinical sensitivity and clinical specificity of RealTime were 98.2% and 89.5% and of hc2 94.7% and 87.7%, respectively. Analytical sensitivity and analytical specificity of RealTime in detecting targeted HPV types evaluated on the largest sample collection to date were 94.8% and 99.8% and of hc2 were 93.4% and 97.8%, respectively. Excellent analytical agreement between both assays was obtained (kappa value 0.84), while analytical accuracy of RealTime was significantly higher than that of hc2. Out of 160 samples with analytically discordant results: 38, 6, 86 and 30 were considered as RealTime true positive, RealTime false positive, hc2 false positive and hc2 true positive, respectively. RealTime displayed high intra-laboratory reproducibility (kappa values 0.98-1.00) and inter-laboratory agreement (kappa values 0.96-1.00) on 500 samples retested between 61 and 226 days after initial testing in two participating laboratories.

**Conclusions:** RealTime can be considered as a reliable and robust HPV assay with comparable clinical specificity and clinical sensitivity as the hc2 in population-based cervical screening settings.

**DETECTION OF HIGH-RISK HPV ONCOGENIC mRNAs AS AN ADJUNCT METHOD FOR CERVICAL CANCER SCREENING; BASELINE RESULTS FOR THE CLINICAL EVALUATION OF APTIMA HPV mRNA (CLEAR) US CLINICAL TRIAL**

Wright T1, Stoler M2, Dockter J3, Reid J3, Getman D3, Giachetti C3

1 Columbia University, USA.; 2 University of Virginia, USA; 3 Gen-Probe Incorporated, USA

**Objective-** To determine the clinical performance of the APTIMA® HPV (AHPV) assay, which detects HPV E6/E7 mRNA from 14 high-risk genotypes, as an adjunctive method for cervical cancer screening of women ≥30 years of age with normal Pap cytology results.

**Methods-** In a pivotal, prospective clinical trial, 13,489 women were enrolled from 19 U.S. sites. Of these, 10,871 women were ≥30 years of age with negative cytology and were eligible for baseline analysis. ThinPrep Pap specimens were tested with both AHPV and the Hybrid Capture 2 (HC2) assay. Women testing positive with either assay (n=846), and a subset of randomly-selected women negative for both (n=556), were referred to colposcopy. Of the1402 women referred, 865 (61.7%) underwent colposcopy with ECC; additional 2mm punch biopsies were obtained from all visible cervical lesions. Disease ascertainment was achieved by consensus histology review. Eligible women who did not attend colposcopy (n=9718) or those diagnosed as <CIN2 (n=827) continued in a 3-year follow-up study.

**Results-** Among the 10,871 women ≥30 yrs with normal cytology, 540 (5.0%) were positive by the AHPV assay and 666 (6.5%) of 10,314 were positive by HC2. Of the 819 women with a consensus histology diagnosis, 20 (2.4%) had ≥CIN2, including 8 cases of CIN3 and 3 cases of adenocarcinoma in situ. Relative risk estimates calculated from absolute risks for ≥CIN2 and ≥CIN3 in AHPV-positive vs. AHPV-negative women were significantly greater than unity and similar to the relative risk estimates calculated for HC2. The false positive rate of AHPV for ≥CIN2 was significantly lower compared to HC2. The true positive rate of AHPV for ≥CIN2 was not significantly different from HC2.

**Conclusions-** These results support the use of HPV oncogenic mRNAs of the 14 HPV high risk types as markers for cervical disease, and demonstrate the clinical utility of the AHPV assay as an adjunctive method in cervical cancer screening.
Objective- To validate the clinical performance of the APTIMA® HPV (AHPV) assay as a triage method for women with ASC-US cytology diagnosis.

Methods- In a pivotal, prospective clinical trial, 1345 women with ASC-US cytology diagnosis from routine Pap testing who met the study criteria were enrolled from 19 U.S. sites. Primary ThinPrep liquid Pap specimens were tested with both the AHPV assay and the Hybrid Capture 2 (HC2) assay. Of these, 1009 women aged 17-71 years had valid AHPV results and conclusive disease status based on adjudicated consensus histology review of 2mm punch biopsies from each of 4 quadrants (randomly obtained at the transition zone if lesions were not visible) and an ECC biopsy. Valid HC2 results were obtained from 930 women in this group.

Results- Of 1009 enrollees, 97 (9.6%) had ≥CIN2 and 43 (4.3%) had ≥CIN3, respectively. The clinical sensitivity estimates for AHPV were similar to those of HC2 (P=0.7266 for CIN2+ detection and P=1.0000 for ≥CIN3 detection). AHPV had fewer false-positive results than HC2; AHPV specificity was significantly higher than that of HC2 for the <CIN2 (P<0.0001) and <CIN3 (P<0.0001) endpoints. NPVs and PPVs were similar between the AHPV and HC2 assays. For AHPV, the lower limit of the 95% CI for PPV was greater than the prevalence, and similarly, the lower limit of the 95% CI for NPV was greater than 100% minus prevalence, indicating the AHPV assay has diagnostic value.

Conclusions- These results support the use of HPV oncogenic mRNAs of the 14 HPV high risk types as markers for cervical disease, and validate the clinical utility of the AHPV assay for triage of women referred with ASC-US cytology results.

Objectives: BD Diagnostics has developed a new automated real-time PCR assay (“BD Viper HPV assay”) that simultaneously detects 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and can provide genotyping information on 7 of these types (16, 18, 31, 45, 51, 52, 59) without the need to perform a reflex test. We tested a randomized convenience sample of 500 cervical specimens collected in the U.S. National Cancer Institute’s ASCUS/LSIL Triage Study (ALTS), which had been stored at -80°C in digene STM medium (Qiagen),using the BD Viper HPV assay. The clinical performance parameters of the BD Viper HPV assay for detection of CIN3+ were calculated and compared to Hybrid Capture 2 (Qiagen).

Methods: The assay utilizes a novel single-step direct chemical lysis method that can process both BD SurePath™and ThinPrep® PreservCyt® (Hologic, Inc.) liquid based cytology specimens (LBC) or a co-collected cytobrush specimen using Ferric Oxide (FOX™) particle DNA binding and magnetic extraction. The sample input volume of the STM specimens for the BD HPV assay was reduced to accommodate the parameters of the clinical cut-off that was used for LBC specimens. BD Viper HPV test results were compared retrospectively to CIN3+ histology results and to the digene High-Risk HPV HC2 DNA Test (Qiagen) results from the original clinical trial.

Results and Conclusions: The BD Viper HPV test had a similar sensitivity and specificity for CIN3+ to that of the hybrid capture assay:

<table>
<thead>
<tr>
<th>CIN3+</th>
<th>Enrollment Colposcopy (n=190)</th>
<th>Worst Outcome Over The Two Year Duration (n = 473)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BD Viper HPV</td>
<td>HC2</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95.7%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>29.3%</td>
<td>23.4%</td>
</tr>
<tr>
<td>PPV</td>
<td>15.7%</td>
<td>14.7%</td>
</tr>
<tr>
<td>NPV</td>
<td>98.0%</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

In addition to its excellent clinical performance, the new BD HPV assay offers a number of advantages including a more streamlined automated workflow, a 5- to 8-fold reduction in sample input volume and the ability to simultaneously genotype high-risk types responsible for > 90% of cervical cancers.
CELLULAR QUANTIFICATION OF HPV E6, E7 mRNA IMPROVES DETECTION OF HIGH GRADE CERVICAL LESIONS COMPARED TO HPV DNA.

Petros KARAKITSOS

Background. HPV DNA is used as an adjunct to the Papanicolaou smear to improve the sensitivity for detection of high-grade cervical lesions (CIN 2+). Though sensitive for CIN 2+, HPV DNA has low specificity resulting in a high clinical false positivity rate. In this study, we used ultrasensitive fluorescence in-situ hybridization/flow cytometry to screen cervical samples for the overexpression of HPV E6 and E7 mRNA (HPV Oncotect®) and compared the performance of this assay with an HPV DNA array for the detection of high grade cervical lesions.

Methods. A total of 1276 women were enrolled in the present cohort study. The cervical samples were analyzed for HPV DNA by clinical arrays and the overexpression of E6 and E7 viral oncogenes was monitored using the HPV Oncotect® E6, E7 mRNA detection kit that quantifies the intracellular HPV E6 and E7 mRNA on a cell-by-cell basis.

Results. HPV Oncotect® positivity increased with severity of lesions, from 29.6% in HPV cases, to 34.6% in CIN 1, 81.8% in CIN 2, 87.5% in CIN 3 and 100% in carcinomas. Samples with a negative HPV Oncotect® test had a post test probability of 1% of having CIN 2+ given the prevalence of a positive test observed in our population (0.059%). A combination of a positive HPV Oncotect® test and high grade cytological lesion had a post test probability for high grade histological lesions of 85% (95% CI: 75-92) and a positive predictive value (PPV) of 99.9% for CIN 2+

Conclusions. E6, E7 mRNA Oncotect Kit provides an early predictor of persistent HPV infection and may improve cervical cancer screening by increasing the specificity of detecting high grade lesions.
**COMPETITION OF VACCINE AND NON-VACCINE HPV TYPES BEFORE AND AFTER MASS-VACCINATION**

Lehtinen M\(^1\), Palmroth J\(^2\), Merikukka M\(^2\), Kaasila M\(^2\), Apter D\(^3\), Paavonen J\(^4\).

1 University of Tampere, 2 National Institute for Health&Welfare, 3 Family Federation of Finland, 4 University of Helsinki

**Objectives:** Replacement of multivalent vaccine covered serotypes of pneumococci by non-vaccine serotypes is abolishing effectiveness of pneumococcal mass vaccination. To understand the likelihood of type-replacement following vaccination against human papillomavirus (HPV) types 16/18 we have studied competition of genital HPV types by assessing multiple infections caused by seven most common genital HPV types 6,11,16,18,31,33, and 45 in fertile-aged Finnish females between 1995-2004.

**Methods and Results:** First trimester serum samples from two consecutive pregnancies (mean 2.5 years apart) were retrieved for a random 3 100 subsample of 123 000 women belonging to the Finnish Maternity Cohort. 42% had antibodies to at least one HPV type at the baseline. Highly significantly increased incidence rate ratios (IRR) of seroconversion to another HPV type were consistently noted for HPV type 33 only, in both HPV16 and HPV18 antibody-positive women: HPV16 antibodies ? 16 and 33 antibodies (IRR 3.2 95% CI 2.0-5.2), and HPV18 antibodies ? 18 and 33 antibodies (IRR 3.6, 95% CI 2.1-5.9); irrespective of the presence of antibodies to other HPV types at baseline: HPV16 antibodies only ? 16 and 33 antibodies (IRR 2.9, 95% CI 1.6-5.4) and HPV18 antibodies only?18 and 33 antibodies (IRR 2.5, 95% CI 1.1-6.0). This suggested competitive advantage for HPV33 over the other genital HPV types before the era of HPV mass vaccination.

To explore type-replacement related to HPV mass vaccination using a prophylactic HPV16/18 virus-like particle vaccine (Cervarix™) with documented cross-protective efficacy against HPV types 31/45, we assessed whether the IRR of non-vaccine HPV types were significantly different in HPV16/18 vaccinated women as compared with hepatitis A-vaccine recipients during a 36 month follow-up. In a sizeable phase III (PATRICIA) trial sub-cohort of initially 4808 16-17 year-old Finnish women the HPV16/18 vaccine coverage ranged between <1% and 20% by age-cohort and study community. The IRR estimates of new HPV types acquired during the follow-up in baseline HPV16 or HPV18 positive individuals as compared to baseline HPV16 or HPV18 negative individuals will be presented.

**Conclusions:** Our studies are the first attempt for addressing the issue of possible HPV type-replacement before and after HPV mass vaccination with considerable vaccine coverage. Surveillance of the phenomenon will continue in a community randomized phase IV trial with 50% vaccine coverage among early adolescent females (11 communities) and females and males (11 communities) compared to hepatitis B-vaccinated early adolescent females and males (11 communities).

**REPEAT HPV TESTING VS. CYTOLOGY TRIAGE OF WOMEN FOLLOWING A SINGLE HR-HPV DNA TEST**

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**Objectives:** HR-HPV DNA testing has shown increased sensitivity and negative predictive value compared with a single Pap smear. However, the relative specificity of HR-HPV DNA testing is lower than cytology, thus the overall balance between benefits of a more sensitive test and harms from over-referral is uncertain. We evaluated 3 alternatives to referral following a single HR-HPV test result by estimating sensitivity and specificity of cumulative 2-year incident CIN2+ from the ALTS study: 12- and 24-month repeat HR-HPV detection, and single HR-HPV testing plus abnormal cytology.

**Methods:** Using a nested case-control design, we selected 325 women with a diagnosis of incident CIN 2+ as cases and a random sample of 401 women with <CIN 2 as controls. HPV DNA status was assessed using hc2 and Linear Array (LA) at enrollment and at each 6-month follow-up visit for a maximum of 2 years. The relative sensitivity and specificity of single vs. repeat measurement of HR-HPV by each testing method was compared using a marginal regression model.

**Conclusions:** Compared with referral after a single HR-HPV positive test result, referral based on repeat detection at 12-months of any HR-HPV by hc2, any HR-HPV by LA, or any type-specific HR-HPV by LA was significantly more specific (specificity ratio and 95% CI: 2.2 [1.9, 2.5], 1.8 [1.6 – 2.0], and 2.0 [1.8, 2.3], respectively), but slightly less sensitive (sensitivity ratio and 95% CI: 0.92 [0.88, 0.95], 0.92 [0.89, 0.97], 0.87 [0.83, 0.92], respectively). There was no advantage in clinical performance in measuring HPV viral persistence for detection of CIN2+ using HPV genotyping vs. repeat positivity for a pool of carcinogenic HPV. The relative changes in sensitivity and specificity of repeat HPV detection increased further when the testing intervals were increased from 12 to 24 months. Similar performance was observed using a CIN3+ endpoint. These data provide useful estimates for evaluation of the impact of sensitivity/specificity trade-offs for three feasible HPV triage strategies.
EVALUATION OF THE HPV16/18 TRIAGE STRATEGY FOR HRHPV-POSITIVE WOMEN AGED≥30 YEARS WITH NILM CYTOLOGY: ATHENA BASELINE RESULTS

Wright Jr, TC1, Sharma A2, Apple R3, Behrens C2, and Wright TL2

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2. Roche Molecular Diagnostics, Pleasanton, CA, USA

Objective: Although clinical management guidelines recognize the utility of HPV16 and 18 genotyping as a triage to colposcopy for high-risk (hr) HPV-positive women aged ≥30 years who are cytologically normal (NILM), there are limited data on how such a strategy would perform in a screening setting.

Methods: The ATHENA HPV study is a randomized trial designed to evaluate testing for pooled hrHPV as well as HPV16 and HPV18 genotyping using the newly introduced cobas® 4800 HPV Test, among women undergoing cervical cancer screening. In total, 47,208 U.S. women aged ≥21 years were enrolled and cervical samples were collected into PreservCyt media for both cytology and hrHPV DNA testing by multiple assays. The cobas 4800 HPV Test can report positive or negative results for 14 hrHPV genotypes, or give three simultaneous HPV results for 12 other hrHPV, HPV16 and HPV18 using real-time PCR technology. Colposcopy with biopsy was performed in all women with ≥ASC-US (equivocal or borderline) cytology results, hrHPV-positive women aged ≥25 years, and a subset of women aged ≥25 years negative by both cytology and hrHPV testing, to determine the presence of cervical intraepithelial neoplasia grade 2 or greater (≥CIN2). Disease status was determined by central pathology review of all biopsies.

Results: Among 32,260 women aged ≥30 years with negative cytology results, the prevalence of pooled hrHPV using the cobas 4800 HPV Test was 6.7%, with a prevalence of 1.0% and 0.5% for HPV16 and 18, respectively. Estimated absolute risk of ≥CIN2 in HPV16/18-positive women with negative cytology was 11.4% (95% CI 8.3, 14.7%) compared with 4.6% (95% CI 3.5, 5.7%) if positive for 12 other hrHPV genotypes and 0.8% (95% CI 0.3, 1.5%) if hrHPV negative.

Conclusions: Genotyping for HPV16/18 using the cobas 4800 HPV Test identifies a subset of women aged ≥30 years with a very high risk for ≥CIN2 missed by cytology. Women with normal cytology who are HPV16 and/or 18 positive share a similar absolute risk of ≥CIN2 and ≥CIN3 in ATHENA as do women with ASC-US who are positive for pooled hrHPV. This suggests that both groups of women should be managed similarly.

AGE-DEPENDENT VARIATION IN HPV TEST PERFORMANCE FOR ≥CIN2 DETECTION IN WOMEN WITH ASC-US: AN ATHENA TRIAL UPDATE

Stoler M1, Sharma A2, Behrens C2, Apple R3, and Wright TL2

1. University of Virginia Health System, Charlottesville, VA, USA - 2. Roche Molecular Diagnostics, Pleasanton, CA, USA

Objective: The evaluation of the cobas® 4800 HPV Test performance for detection of ≥CIN2 disease in women with ASC-US (equivocal or borderline) cytology was an ATHENA primary objective. However, as the frequency and positive predictive value (PPV) of ASC-US is known to vary with age, an age-stratified analysis was conducted to further characterize test performance.

Methods: A total of 47,208 women aged 21 years presenting for routine cervical screening were recruited across the USA. LBC and HPV testing was performed on all cervical specimens. All women with ASC-US cytology were invited to a second study visit for colposcopy with biopsy and/or ECC. All biopsies were adjudicated by a Central Pathology Review Panel (CPRP). Analysis was based on a positive or negative hrHPV test result and ≥CIN2 CPRP histology as the disease endpoint. The performance of the cobas 4800 HPV Test was stratified by age and evaluated for sensitivity, specificity, PPV and NPV. Results were compared to those obtained with hc2 in the same population.

Results: In total, 1,923 women had ASC-US cytology (4.1% of enrolled population) and 1,578 women underwent colposcopy and had valid biopsy results. Mean age of the 1,578 women was 37.1 years (± SD 11.3 years). ASC-US prevalence declined with age, being 5.5%, 4.1%, and 3.6% in the 21–29, 30–39, and ≥40 years strata, respectively. The age-stratified performance of hc2 for detecting ≥CIN2 was similar to the cobas 4800 HPV Test described below:

<table>
<thead>
<tr>
<th>Statistic</th>
<th>21–29 Years</th>
<th>30–39 Years</th>
<th>≥40 Years</th>
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<tbody>
<tr>
<td>N (% of ASC-US)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity, (n/N)</td>
<td></td>
<td></td>
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<tr>
<td>Specficity, (n/N)</td>
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<td>PPV, (n/N)</td>
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<td>NPV, (n/N)</td>
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<td>Specificity, (n/N)</td>
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<td>PPV, (n/N)</td>
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<tr>
<td>NPV, (n/N)</td>
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</table>

Conclusions: While ASC-US interpretation declined minimally across age groups, the prevalence of hrHPV declined more acutely, being >3x as high for ASC-US subjects aged 21–29 years compared to those ≥40 years. Likewise, the fraction of ≥CIN2 detected was highest in ASC-US subjects <40 years, with >5% detected in the 21–29 years stratum. Hence, the predictive value of ASC-US cytology declines dramatically with age once hrHPV presence is accounted for.
**CLINICAL PERFORMANCE OF THE DIGENE HPV GENOTYPING PS TEST FOR THE DETECTION OF HPV 16, 18, AND 45**

**Griesser H.**, Thai H.  
1 ZPZ, Emil-Hoffmann-Straße 7a, D-50996 Köln, GERMANY  
2 QIAGEN Gaithersburg Inc., 1201 Clopper Road, Gaithersburg, MD 20878 USA

**Objectives:** To evaluate the performance of the digene HPV Genotyping PS Test (PS) with clinical specimens collected in both QIAGEN Specimen Transport Media (STM) and Hologic PreservCyt® media (PC). Approximately 520 specimens were included in the Performance Evaluation (PE) with testing being executed across four independent sites in two countries, Germany and Belgium.

**Methods:** Evaluation of 520 clinical specimens was to be included in the PE study with the intention that half were to be STM specimens and half were to be PC specimens. All the specimens included in the study were first characterized as being either HPV high risk positive or negative using the digene HC2 High-Risk HPV DNA Test (HC2). It was determined for the PE study that over 90% of the clinical specimens evaluated were to be HC2 positive as it would demonstrate the capabilities of the PS test as a reflex test for HPV screening positive results. A much smaller percentage of HC2 negative specimens were also tested to evaluate specificity of the PS test. Testing occurred independently across four diagnostic labs located in two countries, Germany and Belgium, with the intention that each lab evaluated an equal proportion of specimens. A QIAGEN in-house validated sequence specific qPCR method for the detection of HPV 16, 18, and 45 was used as the reference method. The reference testing was executed at QIAGEN Gaithersburg, Inc.

**Conclusions:** The PS test leverages the HC2 Hybrid Capture® technology and utilizes QIAGEN’s proprietary RNA probes and hybrid-specific antibodies for the detection of HPV 16, 18, and 45. The familiar platform could offer current HC2 users a manageable HPV genotyping solution for three of the most prevalent HPV genotypes. We demonstrated, through the performance evaluation, good overall concordance of the PS test to qPCR, our reference method. Overall, a total agreement of over 90% was achieved between PS and qPCR highlighting the capability of the PS test to detect HPV infections from both STM and PC cervical specimens at the clinically relevant sensitivity of 5000 copies per assay. Results from STM and PC specimens were comparable demonstrating equivalent detection from either media types. The applications presented here are currently for research use only and are not to be used for diagnostic procedures.

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**EVALUATION OF THE APTIMA® HPV 16 18/45 ASSAY ON THE FULLY AUTOMATED TIGRIS® DTS® AND PANTHER® SYSTEMS**

**Eaton B.**, Clad A., Sikhamsay N., Quinto J., Telebrico M., and Dockter J.  
Gen-Probe Incorporated, San Diego, CA, USA

**Objective:** The APTIMA HPV (AHPV) Assay is a CE-marked multiplex screening assay that detects HPV E6/E7 mRNA from 14 high-risk (HR) types. The APTIMA HPV 16 18/45 Genotype (AHPV-GT) Assay, which is currently being developed, specifically detects HPV types 16, 18, and 45 and differentiates type 16 from types 18 and 45. The AHPV-GT assay is intended for testing AHPV assay positive samples to determine if types 16, 18 or 45 are present. Both assays may be run on the fully automated TIGRIS DTS system and the fully automated PANTHER system. In this preliminary evaluation of assay performance, the analytical limit of detection of the AHPV-GT assay was determined on both instrument systems. Additionally, an assessment of clinical performance of the AHPV and AHPV-GT assays on the TIGRIS and PANTHER systems was performed using liquid Pap specimens.

**Methods:** The analytical limit of detection was determined by testing serial dilutions of HPV 16, 18, and 45 in vitro transcripts prepared in GEN-Probe Specimen Transport Media (STM). Probit analysis was performed to determine the 95% detection limit of each HPV type. The clinical performance of the AHPV Assay on each instrument system was evaluated by testing liquid Pap specimens (n=340) from a referral population, using histology diagnosis of CIN2+ as the endpoint. AHPV positive specimens were then tested with the AHPV-GT assay on each instrument system. Agreement between systems was determined.

**Conclusion:** The 95% detection limit of the AHPV-GT assay for HPV 16, 18 and 45 was comparable on both instrument systems and similar to the 95% detection limit of the AHPV assay for these genotypes (< 50 copies/reaction for each type). The sensitivity of the AHPV assay for detection of CIN2+ was also comparable on both instrument systems (McNemar’s P-value of 0.5637). The positive, negative and overall agreement for the TIGRIS and PANTHER systems with the AHPV assay was 95.2%, 96.1% and 95.6%, respectively. Of the AHPV positive specimens on the TIGRIS system (n=186), 50.0% were positive with the AHPV-GT assay. Of the AHPV positive specimens on the PANTHER system (n=183), 51.4% were positive with the AHPV-GT assay. Approximately 90% of the specimens that were AHPV-GT positive were differentiated as HPV 16 positive on both instrument systems. In summary, the TIGRIS and PANTHER systems yield comparable analytical performance for detection of HPV 16, 18 and 45 with the AHPV-GT assay. The instrument systems also yield comparable clinical performance and demonstrate strong agreement for detection of high-risk HPV in liquid Pap specimens.
RISK MANAGEMENT AND ASSESSMENT USING HPV GENOTYPING

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Carcinogenic human papillomavirus infections are the necessary cause of almost all cervical cancers. HPV infections are very common and most infections regress spontaneously within a few months. HPV16 and HPV18 cause about 73% of cancers worldwide; another 21% of cancers are caused by HPV58, 33, 45, 31, and 52. Due to the frequent occurrence of multiple HPV infections, estimating the attribution of carcinogenic HPV types to high-grade CIN is more complicated. Long-term prospective studies have shown a substantially different risk of developing CIN3 and cancer for different carcinogenic HPV types, with HPV16 and HPV18 being the types with highest risk. Based on these findings, HPV genotyping has been proposed as a triage test for HPV-positive women. While genotyping may offer important additional risk stratification beyond simple HR HPV DNA testing, implementation into screening programs is not trivial. Clinical use of HPV genotyping requires well-validated high quality assays approved by regulatory authorities. The gain in risk stratification from HPV genotyping needs to be high enough to influence management.

GLOBAL REDUCTION OF CERVICAL CANCER WITH HPV VACCINE: INSIGHTS FROM THE HEPATITIS B VACCINE EXPERIENCE

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Development of safe and effective vaccines against human papillomavirus (HPV)—the second vaccine against a major human cancer—is one of the most important medical and public health achievements of this century. As with all new vaccines, HPV is currently expensive and this cost precludes its use in the developing world, which has the greatest burden of disease from HPV-related cancers. Hepatitis B (HB) virus vaccine, which prevents chronic HB infection and related cirrhosis and liver cancer, has been successfully introduced as a routine vaccine for children in 89% of countries, including the poorest. The success of this vaccine provides a model for the introduction of HPV vaccine and control of cervical and other HPV-related cancers and genital warts. Lessons learned from HB vaccine introduction are relevant to our efforts to introduce HPV vaccine globally. As with HB vaccine, introduction of HPV vaccine into national immunization programs and routine use of this vaccine, funded by governments, will be needed to control HPV-related disease on a global basis. Global funding support will be needed to make control a reality for the poorest countries, and the program to accomplish this, the Global Alliance for Vaccines and Immunization (GAVI), has already expressed great interest in including HPV vaccine although they will need to raise more money to do so. In addition, the manufacturers will need to dramatically reduce the vaccine price for the poorest developing countries, which they have committed to do, and must tier prices for higher income developing countries not eligible for GAVI support. Countries will need to decide on the priority of HPV control in the context of other important new vaccines against pneumococcal pneumonia and rotavirus diarrhea. Although infant immunization rates are high in even poor developing countries, immunization of older children, adolescents, and young adults will require development of additional infrastructure. HPV immunization must also be implemented as part of a comprehensive control program that includes immunization, screening, and treatment of disease.
METHODS TO MONITOR THE IMPACT OF HPV VACCINATION PROCESS IN HIGH, MIDDLE, AND LOW RESOURCE COUNTRIES

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Objectives: To review methods to monitor HPV vaccine program impact at a population level and to summarize progress in monitoring biologic impact in several countries.

Findings: Since HPV vaccines were registered in 2006, several high income countries have begun to monitor the biologic impact of their HPV vaccination programs and a few low- and middle-income countries are planning monitoring projects. Projects are assessing many outcomes outside the context of controlled trials: immune response to standard and alternate schedules, herd immunity, HPV prevalence, and the incidence of precancerous lesions, anogenital cancer, and warts. Because the immunologic correlates of vaccine protection are not well understood and many countries lack organized screening programs that could track precancerous lesions, measuring HPV prevalence (shortly after onset of sexual activity) and the incidence of warts and cancer incidence may be the most feasible and least biased ways to assess short- and long-term impact. Some projects demand novel collaborations between immunization, STI, and cancer programs, biobanking, or newly linked health information systems. Early results are promising, including evidence that alternate 3 dose schedules and 2 dose regimens invoke strong immune responses and that quadrivalent HPV vaccine introduction in Australia has resulted in a substantial decline in wart incidence.

Conclusions: Monitoring the impact of HPV vaccine programs is more complex and lengthy than that for other vaccines. Methods vary according to program design and the financial, clinical, laboratory, and surveillance resources that are available on a large-scale, long-term basis. Monitoring methods may also change as HPV testing and cervical cancer screening methods evolve. Results of projects that evaluate biologic outcomes should be considered along with data on post-marketing vaccine safety and coverage to guide the planning and budgeting of immunization, cancer, and STI programs and to estimate program health impact and cost-effectiveness. Long-term advocacy and financing is especially needed to monitor new vaccine programs in low-income countries where vaccines promise to substantially reduce cancer incidence and mortality.

EARLY BENEFITS AND PREVENTION OF ABNORMAL CERVICAL SMEARS WITH THE BIVALENT HPV VACCINE

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Background: The ASO4-adjuvanted HPV-16/18 vaccine (HPV2) demonstrates high prophylactic efficacy against precancerous lesions associated with oncogenic HPV types. However an important and additional benefit would be a reduction in abnormal cervical cytology and a reduction in colposcopy referrals and cervical excision therapies. Reduction of these abnormalities may translate into healthcare cost savings.

Methods: Presentation of data from available studies documenting these results.

Results: End of study results from the PATRICIA trial (median follow-up 47.4 months) in the total vaccinated cohort-naïve reported an efficacy of 91.9% against the development of ASCUS+ associated with HPV-16/18. Vaccine efficacy against ASCUS+ associated with the 10 most common non-vaccine HPV types and irrespective of HPV type, was 24.0% and 23.2% respectively. The reduction in colposcopy referrals was 29.0% with reductions in cervical excision therapies of 70.2%.

Conclusion: Vaccination of HPV naïve women approximates the target population of public health organized vaccination programs. Administration of HPV2 results in a reduction in abnormal cytology with a corresponding reduction in colposcopy referrals and in cervical excision procedures. Reducing these abnormalities may result in healthcare cost savings.
SS 3-4

EARLY BENEFITS AND PREVENTION OF ABNORMAL SMEARS – QUADRIVALENT VACCINE
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Objectives: To describe changes in the incidence and prevalence of high grade cervical lesions in young Australian women since the commencement of the National HPV Vaccination Program.

Methods: In Australia, cervical screening using conventional cytology is recommended every two years commencing at age 18, or two years after first sexual intercourse (whichever is later). Between 2007 and 2009, quadrivalent HPV vaccine was offered to all Australian women aged 12 to 26 years, meaning that there was an immediate overlap between vaccinated and screened women. The median age of first sexual intercourse in Australia is 16 years. Monitoring of the National Cervical Screening Program indicators (which include participation, cervical abnormality rates and cervical cancer incidence and mortality) is conducted through analysis, at the national level, of data from Australia’s state based Pap Test Registers and Cancer Registries. State based Pap Test Registers also conduct their own research and analysis of screening data. I will present the latest incidence and prevalence data from the Victorian Cervical Cytology Register, which is already recording declines in high grade cervical abnormalities amongst young women.

Conclusion: Population level data indicate that HPV vaccination may already be having an impact on cervical abnormality rates in Australia. The decline in high grade abnormalities is likely to increase with time, as women vaccinated prior to sexual debut enter screening, but the maximal impact that will be achieved is uncertain given that vaccination cannot prevent all high grade abnormalities and that the extent of any reduction through herd immunity for unvaccinated women is as yet uncertain. In the short term, data linkage between the National HPV Vaccination Program Register and Pap Test Registers needs to be conducted to confirm that the observed declines are indeed due to HPV vaccination.

SS 3-5

REDUCTION IN COLPOSCOPY AND CLINICAL PROCEDURES - BIVALENT VACCINE
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Background and methods
In the PATRICIA Phase III trial, women aged 15–25 years were randomised to receive HPV-16/18 vaccine or hepatitis A vaccine as control at Months 0, 1 and 6. Cervical samples were collected every 6 months for HPV-DNA typing and every 12 months for gynaecological/cytopathological examinations. Women were referred for colposcopy and treatment according to pre-defined algorithms. Vaccine efficacy is clinically of most interest for (1) the total vaccinated cohort (TVC) - women who received ≥1 dose, regardless of baseline HPV DNA/serostatus and (2) for the TVC-naïve cohort - women who received ≥1 dose, were HPV-16/18 seronegative, DNA-negative for 14 oncogenic HPV types, and had normal cytology at baseline. These two cohorts approximate to the general population of women, many of whom will have already been exposed to HPV (TVC cohort) and to the adolescent girls currently included in most vaccination programmes (TVC naïve).

Results: At the end-of-study analysis (median follow-up: 47.6 months for TVC and TVC-naïve), VE (95% CI) against ASCUS+ associated with HPV-16/18 was 68.4% (64.1–72.3; p<0.0001) and 91.9% (88.8–94.3; p<0.0001) in the TVC and TVC-naïve, respectively. For ASCUS+ irrespective of HPV type, VE was 12.2% (7.2–16.9; p<0.0001) and 23.2% (16.9–29.0; p<0.0001), respectively. Corresponding reductions in colposcopy referrals were 14.8% (8.9–20.3; p<0.0001) and 29.0% (21.6–35.8; p<0.0001) with reductions in cervical excision therapies of 33.2% (20.8–43.7; p<0.0001) and 70.2% (57.8–79.3; p<0.0001).

Conclusions: Vaccination with the AS04-adjuvanted HPV-16/18 vaccine reduced abnormal cytology rates and therefore colposcopy referrals and cervical excision therapies in both the TVC and TVC-naïve cohorts. A greater reduction was seen, as would be expected, in excision therapies because HPV 16 and 18 are more prevalent in high grade disease, which requires treatment.
THE IMPACT OF HPV VACCINATION IN REDUCING THE RATES OF LOWER GENITAL TRACT PROCEDURES

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Both HPV vaccines have excellent efficacy in preventing cervical neoplasias, and the quadrivalent vaccine also has excellent efficacy in preventing other anogenital neoplasias including genital condyloma. However, their impact in the reduction of abnormal Pap tests, colposcopic examinations and treatment conveys important societal benefits. The bivalent vaccine reduced the rate of abnormal cytology for ASCUS, LSIL and HSIL by 23.9%, 92.6% and 53.7%, respectively. Rates of colposcopy examinations and cervical excisional procedures were reduced by 26.3% and 68.8%, respectively. The quadrivalent vaccine reduced the rate of abnormal Pap tests by 17.1%, and LSIL by 17.0% and HSIL by 44.5%. Rates of colposcopic examinations, cervical biopsy and cervical surgery were reduced by 19.8%, 22.0% and 42.3%, respectively. Further, the vaccine reduced the rate of procedures for external genital lesions by 43.3%. Actual community-based rates of reduction of procedures are determined by various factors. Other important benefits should be considered.

LONG-TERM EFFICACY OF HUMAN PAPILLOMAVIRUS VACCINATION AGAINST CIN 3 AND INVASIVE CARCINOMA: REGISTRY BASED FOLLOW UP OF A PHASE III TRIAL (FUTURE II)

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Objectives: Human papilloma viruses (HPV) 16/18 are known to cause approximately 70% of cervical cancers. Phase III clinical trials of HPV vaccination have demonstrated >95% efficacy against persistent HPV type 16/18 infections and associated cervical intraepithelial neoplasia (CIN) grade 2+ lesions, and up to 90% efficacy against all CIN3+ lesions. A long-term follow-up is, however, needed to confirm the protective efficacy against cervical carcinoma.

Methods: Phase III clinical trial (FUTURE II) consisted of intensive clinical 4-year follow-up including health education and counselling. The intervention potentially affects the incidence of cervical neoplasia also in the placebo group. In order to increase power of the long-term follow-up to determine the impact of the clinical intervention as such, a population based reference cohort of similarly aged women not exposed to such intervention was enrolled at the same time from the same communities. The HPV vaccine cohort and placebo vaccine cohort of 16-17 year old women from the Finnish FUTURE II trial (N=1,749) and a reference cohort of 18-19 year old women (N=15,744) were linked with the Finnish Cancer Registry to determine the incidence of CIN3 and invasive cervical carcinoma (CIN3+) during the passive follow-up, starting 6 months after the clinical follow-up of the phase III trial was completed (lapse time of reporting given for cases originating from the clinical follow-up). All the linkages were done using personal identity codes.

Results and Conclusions: Currently the incidence of CIN3+ at the age of 20-24 years is 95 per 100,000 person years in Finland (www.cancer.fi). The incidence doubles in 5 to 10 years as the cohorts age. Thus, in less than 10 years the cumulative incidence yields 80% power to demonstrate 90% vaccine efficacy against cervical CIN3+. During the first two years this passive registry-based follow-up identified no CIN3+ cases in the HPV vaccine cohort, 2 cases in the placebo vaccine cohort, and 21 cases in the unvaccinated reference cohort suggesting that the vaccine efficacy translates into efficacy against cervical cancer. The passive follow-up continues and new cases emerging in the future will be monitored by repeating linkages with the population-based cancer register at specific time intervals. Person years will rapidly accumulate in our long-term follow-up study. Person years will rapidly accumulate in our long-term follow-up study. In conclusion, valid comparisons between the vaccine and placebo recipients (excluding cross-vaccinated placebo vaccine recipients) and the reference cohort not exposed to intervention are feasible, and will be critical to define more definitively the long-term protection provided by HPV vaccination against the hard endpoints.
THE CLINICAL IMPACT OF CROSS PROTECTION WITH THE BIVALENT VACCINE

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Background: The human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine has shown high and sustained vaccine efficacy (VE) against infections and cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18 as well as evidence of VE against some non-vaccine oncogenic types.

Objectives: The end-of-study results (Month 48) from the Phase III PATRICIA study with respect to overall and individual cross-protective VE against 6-month persistent infection and CIN2+ are presented.

Methods: In this study (NCT00122681), women aged 15–25 years were randomised (1:1) to receive HPV-16/18 vaccine (N=9319) or control (N=9325) at Months 0, 1 and 6. Cervical samples were collected every 6 months for HPV DNA typing; gynaecological and cytopathological examinations were performed every 12 months. VE results are reported for the total vaccinated cohort (TVC; at least 1 dose, irrespective of HPV DNA/serostatus and cytology at baseline; mean follow-up time 43.7 months post-dose 1) and TVC-naïve (women who received ≥1 vaccine dose, seronegative for HPV-16/18 and HPV DNA negative for 14 oncogenic HPV types, with normal cytology at baseline; mean follow-up time 44.3 months).

Results: Overall VE (95% CI) against CIN2+ lesions irrespective of HPV type was 33.1% (22.2;42.6) in TVC and 64.9% (52.7;74.2) in TVC-naïve, with corresponding VE of 45.6% (28.8;58.7) and 93.2% (78.9;98.7) against CIN3+ lesions. VE against 6-month persistent infection in the TVC-naïve was 77.1% (67.2, 84.4) for HPV-31, 43.1% (19.3, 60.2) for HPV-33 and 79.0% (61.3, 89.4) for HPV-45. VE against CIN2+ was 89.4% (65.5, 97.9) for HPV-31, 82.3% (53.4, 94.7) for HPV-33 and 100% (41.7, 100) for HPV-452.

Conclusions: End-of-study results of PATRICIA confirm that HPV-16/18 AS04-adjuvanted vaccine provides cross-protective efficacy against HPV-31, HPV-33 and HPV-45 in HPV-naïve women. This protection beyond the vaccine HPV types -16 and -18 is expected to contribute to additional and clinically meaningful reductions in the overall incidence of precancerous lesions and cervical cancer.


ANALYSIS OF QUADRIVALENT HPV VACCINE EFFICACY AGAINST HPV 16/18 PERSISTENT INFECTION IN BOTH MEN AND WOMEN

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Background: Previous analyses of quadrivalent HPV (qHPV) vaccine in young women, adult women and men have primarily focused on efficacy against disease (both pre-cancerous and cancerous lesions). However, efficacy data against persistent HPV infection were gathered during some clinical studies of the vaccine, including high-risk HPV types 16 and 18. In this report we present vaccine efficacy against persistent infection with HPV 16/18 in clinical trial populations of young women (16-26 years), adult women (24-45 years), and young men (16-26 years).

Methods: Data presented are from 3,350 young women, 3,127 adult women, and 2,777 young men enrolled in 4 clinical trials of the qHPV vaccine who returned for follow-up. Subjects received vaccine or placebo at day 1, and months 2 and 6. Ascertainment of HPV-related infection was accomplished via anogenital sampling (conducted every 6 months), followed by PCR testing. Persistent infection was defined as detection of the same HPV type (16 or 18) in an anogenital swab or biopsy specimen collected on >2 consecutive visits >6 months (±1 month) apart. Analyses were conducted in a per-protocol population (received 3 doses of vaccine/placebo within 1 year of enrollment, were naïve to the relevant HPV types at day 1, and remained free of infection through the completion of the vaccination regimen).

Results: Vaccine efficacy against HPV 16/18 persistent infection in young and adult women was 99% (95% CI: 96-100) and 86% (95% CI: 69-95), respectively. Efficacy against HPV 16/18 persistent infection in young men was 84% (95% CI: 72-91).

Conclusions: The qHPV vaccine provided robust efficacy against persistent infection with HPV 16/18 in both men and women. While persistent infection can be a precursor to pre-cancerous and cancerous HPV-related lesions, in an era of cervical and anal cancer screening using HPV DNA testing the importance of protection against persistent infection detectable on viral screening should not be underestimated.
LONG TERM PROTECTION AGAINST CERVICAL HPV INFECTION WITH THE BIVALENT HPV VACCINE

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Background: Long term protection after HPV vaccination is critical because women remain at risk of cervical infection throughout their sexually active life. Vaccination programs against cervical HPV infection have been implemented before the full duration of vaccine protection is known. Whether booster doses will be required is unclear at this time. Although an immune correlate of protection has not yet been established, the absence of any suggestion of waning protection provides a reason to be cautiously optimistic that protection will be long term. Predictions of long term protection rely upon efficacy phase 3 studies and mathematical modelling of phase 2 immunogenicity studies.

Methods: Presentation of data from available studies documenting the long term protection of the ASO4-adjuvanted HPV-16/18 vaccine (HPV2).

Results: Immunogenicity and efficacy against infection and cervical lesions associated with HPV-16/18 after receipt of three doses of HPV2 has been demonstrated up to 8.4 years. In this group of women who were HPV 16/18 seronegative and DNA negative for 14 oncogenic HPV types at study entry, all women remained seropositive and demonstrated neutralizing antibodies several folds higher than titers following natural infection. Vaccine efficacy against CIN2+ related to HPV 16/18 remained at 100% at 6.4 years.

In a larger trial of women who were entered regardless of their baseline HPV status, end of study data with up to 4 years of follow-up reported vaccine efficacy against CIN2+ associated with HPV 16/18 of 92.9% in the ATP-E and 98.4% in the TVC-naive. For all CIN2+ lesions irrespective of HPV type, vaccine efficacy was 70.2% in the TVC-naive.

There is also evidence that HPV2 offers cross-protection against some non-vaccine oncogenic HPV types.

Conclusion: The ultimate answer of how long, long term protection will last will only be established with ongoing monitoring but currently available results are very encouraging. Mathematical modelling studies predict that high levels of neutralizing antibodies will last for at least 20 years.

HOW TO OVERCOME BARRIERS TO VACCINATION IN YOUR PRACTICES

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The most important is a lack of public knowledge about HPV and cervical cancer. This often results in women and parents not seeing HPV vaccination as being relevant to themselves or their daughters. Ignorance also fosters negative attitudes and misconceptions.

Unfortunately, this is compounded by lack of knowledge among health professionals. Many doctors find it difficult and embarrassing to raise issues to do with sexuality with their patients. Studies in Asia have found that many, and in particular Muslim physicians felt there were particular difficulties in recommending an STI vaccine to their patients, and preferred to emphasize cervical cancer prevention. Rumours that immunisation is a plot to sterilise girls, or to use them as guinea pigs for vaccine experiments, have seriously damaged immunisation programmes in a number of developing countries in Asia, Africa and Latin America. Anti-vaccine groups are increasingly sophisticated in their use of the media, especially the Internet.

In general, in all countries, health professionals look to professional bodies and government recommendations for support and guidance. Reporting in the media affects both the public and health professionals. Advocacy groups such as Jo’s Trust, ECCA and WVACC have an important role. We should encourage people to discuss vaccination with a health professional, however, we need to ensure that the health professionals are well informed.

Issues such as culture, values and beliefs must be taken into account. Different people may view the same information from different perspectives, as they perceive it actually applies to them. Health professionals should be able to assess their patient’s knowledge and address their concerns. Many people are worried about the safety of vaccines in general, and the possible side effects. These issues should be proactively mentioned, because otherwise people may leave the consultation with prejudices and misconceptions that simply have not been mentioned.
Objectives: To monitor the epidemiology of type-specific HPV infections in order to assess the impact of HPV vaccination, during the 5 years after the introduction of the vaccination programme in England, before the expected impact on cervical disease incidence is observable.

Methods: We are using anonymised residual clinical samples to monitor type-specific HPV prevalence and to monitor vaccine-type seroprevalence in young women. Vaccination status is obtained were possible to estimate vaccine effectiveness. Unvaccinated women are included to give insights into herd-immunity effects. High-risk groups are included to investigate potentially important variation in vaccine-induced protection. Type-specific HPV surveillance of later disease outcomes (cervical pre-cancers and cancers) is being established.

Conclusions: The UK has achieved high coverage of HPV vaccination in cohorts targeted at the routine age (80% of 12-13y females) and in the catch-up cohorts (~50% of 13-18y females). The impact of immunisation on the prevalence of HPV 16/18 in young women should start to be evident amongst catch-up cohorts in 2011/12. An increasing effect should be seen as the younger catch-up cohorts and routine cohorts with higher coverage and lower rates of pre-existing infection begin to enter surveillance. By 2013/14 we expect to also observe the impact of cross-protection and herd-immunity on infection rates at the population level.

In this presentation I will briefly describe the school based HPV vaccination program in Australia including

• Organisational aspects (including staffing, intersectoral collaboration, consent processes, follow up)
• Coverage monitoring
• Management of adverse events
• Ongoing challenges
THE U.S. HPV VACCINE PROGRAM AND MONITORING IMPACT: CURRENT LESSONS LEARNED.

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Background: In 2006, the U.S. Advisory Committee on Immunization Practices recommended the quadrivalent HPV vaccine for adolescent girls to prevent cervical cancer and genital warts. Since then, several notable expansions have occurred including recommendation of the bivalent HPV vaccine for adolescent women, advocating use of the quadrivalent HPV vaccine to adolescent males to prevent genital warts.

Methods: We describe the current U.S. HPV vaccine program, latest HPV vaccine coverage, and briefly review the current monitoring plans and status of evaluations.

Results: The HPV vaccine coverage has increased gradually from 2006 through 2009. In 2009, at least 44.3% of girls aged 13 to 17 years old received at least one dose and 27% 3 doses. Various initiatives are in place to monitor the impact of the HPV vaccine for both proximal and distal outcomes. Current challenges and preliminary results of such efforts will include baseline genotyping results and current efforts to describe population-based incidence of cervical intraepithelial neoplasia, and HPV prevalence.

France has been one of the very first European countries to introduce, in 2007, HPV vaccination into its national immunisation schedule. The target group is made of the 14 years old preadolescent girls. A catch up for older girls and young women up to 23 years of age is also recommended but restricted to those who have not yet started their sexual life or have started it less than a year ago. As for other vaccines targeted to children above 6 years or adults, HPV vaccines are mainly administered by private general practitioners (GP’s) or other private practitioners. As of beginning of 2011, only very few districts or cities within districts have included HPV vaccines in their public vaccination offer. HPV vaccinations performed in the private sector are reimbursed by the social security scheme at a rate of 65 % (70 % for the clinician’s consultation) for the target populations for which it is recommended (both for routine and catch up vaccination); However most people (about 80 %) are covered by a private insurance that bears the remaining share. There is no offer of HPV vaccine in schools. Targeted girls (or women for the catch up) have to go, by their own initiative, in a place where the vaccine is proposed in order to get vaccinated.

In conclusion, the HPV vaccination delivery system in France presents the same advantages and limitations as do other vaccinations: On the one side, the HPV vaccines are both geographically and financially widely available through the possibility of any medical practitioner to prescribe and administer them and the very high proportion of the target population for which they can be obtained at no expenses. On the other side, the still very limited activities of active identification, invitation and follow up of the target population is an impediment to high coverage. As of end of 2009, HPV vaccination coverage (for the full series) estimated through data from the National Drugs Reimbursement Database was 23 % for girls aged 15 years old and between 26 % and 33 % for girls aged 15 to 18 years olds.
SS 4-7  
**HPV VACCINE PROGRAMS AND MONITORING: CURRENT STATUS AND LESSONS LEARNED VOLUNTARY BASED INITIATIVES: ITALY**

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**Background:** Italy introduced the recommendation for immunisation against HPV of all 12-year old girls at the beginning of 2007. However, since the implementation of vaccination programmes is under the responsibility of Regional Authorities, the start of immunisation ranged from July 2007 to November 2008 in the 21 Italian Regions. Seven Regions added a further age cohort of adolescent females to which they offer HPV vaccination free of charge (one of them offered it to 4 age cohorts). In addition, many regions also foresee the possibility to supply the vaccine to age groups not included in the active free of charge offer by the system of co-payment (cost of the vaccine to the Regional Health System plus a little amount for vaccine administration).

**Methods:** Every six months, regional authorities committed themselves to report HPV vaccination coverage to the National Institute of Health based on information collected at the level of each Local Health Unit on the number of first, second and third dose administered over the number of resident female in the involved age cohorts. An inquiry was also performed on the kind of vaccine used in each Region.

**Results:** Overall, in the age group of girls born in 1997, the national coverage with at least 1 dose was 67.7%, with at least 2 doses 64.9%, and with 3 doses 59.1%. However, the coverage was highly variable according to the Region, ranging (3 doses) from 22.5% in the Autonomous Province of Bolzano to 80.4% in Basilicata. A good coverage was reached in the supplemental age groups in the Regions that extended free-of-charge offer (usually above 60% with 3 doses).

**Conclusions:** The average coverage with HPV vaccines in Italy was rather good in the first involved cohorts of females. However, preliminary data on the 1998 cohort seem to indicate a stagnant or even worsening situation. Moreover, the higher coverage in older girls compared with 12-year olds underpins the need to increase efforts to better communicate the risks of HPV infection, and the need to accept the HPV vaccination offer before the start of sexual activity.

**SS 4-8**  
**QUADRIVALENT HPV VACCINATION IMPACT IN PORTUGAL: 2007-2010**

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**Objectives:** Human Papillomavirus (HPV) vaccines are available in Portugal since 2007 in the private market. Inclusion in the National Vaccination Calendar started in October 2008 targeting 13 years old adolescents’ cohort for routine vaccination; a catch-up campaign was set for 17 years old girls from 2008 to 2011.

The quadrivalent vaccine against HPV types 6, 11, 16 and 18 was selected. One million doses of 6/11/16/18 HPV vaccine were distributed in Portugal until December 2010, to supply both Primary Care Centers, responsible for the HPV public vaccination program (totally funded) and pharmacies (private out-of-pocket market).

The purpose of this analysis was to assess HPV vaccination impact in terms of health gains and costs avoided to the healthcare system.

**Methods:** The study undertook the healthcare system perspective and included all direct costs. Indirect costs as productivity loss were not included. An incremental analysis was made comparing pre-vaccine era standard practice of screening versus vaccination and screening. We used a Markov model, developed by Myers and others, that mimics natural history of the disease, from HPV infection progression to cervical cancer, calibrated to the Portuguese data. The cost-effectiveness analysis used TreeAge Pro software. Analytic horizon was life-long, until 85 years of age. The study analysed health and budget impact of vaccinating 14 cohorts of girls and young women. Coverage rates considered were 85 and 80%, respectively, for routine and catch-up cohorts. Sales on private and public markets represent, respectively, around 180,000 and 820,000 vaccines doses. For the private market (18-26 years), a decreasing coverage rate of vaccination with age was also considered to reflect the expected behaviour of young women.

**Conclusions:** Impact resulted in 422 deaths, 2,225 cases of cervical cancer, 24,085 cases of cervical pre-cancerous lesions and 19,352 cases of genital warts avoided. A total of 114 million Euros in costs are expected to have been avoided in disease diagnosis, management and treatment. Of this, 30 million Euros represent costs avoided from cases of cervical cancer, 59 million from cases of cervical pre-cancerous lesions and 11,5 million from cases of genital warts. According to this model quadrivalent HPV vaccination in Portugal resulted in significant advantages in terms of health gains, budget impact and value for money.
THE DANISH HPV PROGRAMME: A SUCCESS STORY – VACCINATION BY GENERAL PRACTITIONERS

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Objective: To present a successful implementation of HPV-vaccination using the existing system for childhood immunization by general practitioners.

Methods: HPV vaccine was introduced in the national immunization programme Oct 2008 with a 2-year catch-up programme for 3 girl’s cohorts born in 1993, 94 and 95 (13-15 years old). Routine vaccination of 12 year old girls was started Jan 2009. Vaccination is state financed and free of charge. The 4-valent vaccine was chosen in a tender process. Focus was on solid planning, information, documentation, transparency, handling adverse events and the message: vaccine for cancer prevention.

An information package was send to the GP’s and school health services, a website www.stophpv.dk was created and afterwards invitation by direct mail was sent to the girls and their parents. In preparation for negative press in connection with timely coincidence of adverse events, a baseline study of incidence of autoimmune disease was made. A coordinated response to the press by the authorities was set up. Details will be discussed; the latest coverage data, measures to improve coverage and the vaccination register will be presented.

Conclusions: Coverage for the 3 cohort catch-up programme is above 85% for 3 doses HPV vaccine which is higher or comparable to school based programmes. School vaccination is generally recommended for HPV-vaccination, but the Danish experience shows a successful implementation using general practitioners.

MANAGEMENT OF VULVAR NEOPLASIA

Michel Roy MD, FRCS

Vulvar cancer represents about 1% of cancers in women and 5% to 8% of cancers of the genital tract, about 1.5 to 2 per 100,000 women. The incidence has been reported to rise recently, mostly because of the frequency of HPV and VIN in younger women. The mean age of patients with vulvar cancer used to be after the menopause, but in the last years, it is reported to be around 50 years. Even close to 25% are reported in patients before the age of 40.

Most of the time, the diagnosis is made after a punch biopsy of an exophytic lesion. When the patient is young, invasion is often found after the excision of lesion thought to be “intra-epithelial” on biopsy. In older patient, it is frequently linked with non treated lichen sclerosus.

Management of invasive vulvar cancer is dependent on lymphatic drainage: radical local excision and evaluation of the inguinal lymph nodes. Nowadays we try to be as conservative as possible. The “en bloc dissection” of the whole vulva and inguinal lymph nodes has been replaced by partial vulvectomy and sentinel lymph node excision. When the cancer is lateralized and the sentinel node is unilateral, one can even be more conservative. When the sentinel node is negative for cancer metastasis, complete inguinal lymphadenectomy can usually be avoided in order to significantly lower the incidence of inguinal lymphocysts and lymph oedema of the legs. Such complications are present in close to 90% of patients treated with classical radical vulvectomy and inguinal node dissection.

In rare cases, radiation therapy and chemotherapy can be used for primary or adjuvant treatments.
THE ROLE OF COLPOSCOPY IN THE EVALUATION OF VULVAR DISEASE

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Objective: The aim of this presentation is to discuss the controversy surrounding the role of colposcopy in evaluating the vulva in general, and in cases with VIN in particular.

Methods: For that purpose we have reviewed guidelines, publications and obtained expert opinions.

Findings: There is a general agreement that colposcopy is essential for studying the cervix in cases of abnormal pap test. Cervical colposcopy concentrates on depicting acetowhite epithelium and abnormal vascular patterns in the transformation zone after the application of 3%-5% acetic acid. However, on the vulva, there is no transformation zone, and the vascular patterns associated with intraepithelial neoplasia are less distinct. Furthermore, the opponents of colposcopy of the vulva (vulvoscopy) believe that the whitening seen with acetic acid is common in many healthy women, leading to false positive results, especially in nonexperienced eyes. However, several experts found that once a lesion is discovered or suspected, a colposcope is a useful tool to characterize the lesion and outline its margins. The studies quoted as showing a poor correlation between vulvar acetowhitening and underlying pathology were performed in healthy asymptomatic population. Consequently, some accept that the colposcope is not advantageous over a simple magnifying glass in screening asymptomatic women for vulvar lesions. However, the use of a 5% acetic acid solution to the vulva followed by colposcopy may clearly demarcate dense epithelial acetowhite areas. On the mucosal surfaces, abnormal vascular patterns, like mosaicism and punctuation can be depicted. The acetic acid transforms a subtle focus of VIN to a clearly acetowhite epithelium. It is also useful in evaluating the margins of the lesion and planning excisional treatment or vaporization by CO2 laser. Even as a magnifying tool, the colposcope is powerful as it has several magnifications, a good light source and usually an attached camera or video recorder. An additional advantage of having the colposcope at the vulvar clinic is that examination of a patient with VIN should also include cervix, vagina and anus, since multicentric disease has been reported in patients with VIN. Up to 50% of women with VIN will have antecedent or concomitant cervical, vaginal or anal intraepithelial neoplasia.

It should also be considered that apart from the colposcope there are only few other tests assisting the clinician in diagnosing vulvar disease. While for evaluation of the cervix one can use cytological evaluation, HPV-DNA analysis, colposcopy with application of acetic acid and using a green filter, biopsy and loop conization. For the vulva, it is only the visualization of the lesion and biopsy.

Conclusion: colposcopy has a definite role in evaluating the vulva, although its drawbacks should be taken in account.

IMPACT OF VACCINATION

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Objective: To review efficacy data of HPV vaccines for the prevention of vulvar disease

Methods: Review of available data

Conclusions: Genital warts and vulvar intraepithelial neoplasia (VIN) is caused by HPV. More than 80% of genital warts are caused by HPV 6 or 11. About one third harbors oncogenic HPV strains such as HPV 16 or 31. Quadrivalent HPV vaccine has been demonstrated to prevent 99% of genital warts caused by the four vaccine types. Recent population based data from Australia have shown that a vaccination program with a coverage of >80% among girls and young women has led to a reduction by 2 thirds within 2 years. In males, who are not part of the vaccination program, a 30% reduction was observed, this is the first demonstration of herd immunity for an HPV vaccine. Low grade VIN is mainly caused by HPV 6, high grade VIN mainly by HPV 16, 31 and 6. Data from the quadrivalent HPV vaccine have demonstrated a 100% prevention of VIN caused by the four vaccine types.
ADJUNCTIVE USE OF A NOVEL HPV ISH ASSAY SIGNIFICANTLY IMPROVES DIAGNOSTIC ACCURACY IN CERVICAL BIOPSY INTERPRETATION

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Objectives: The interpretation of cervical biopsies by pathologists is well documented to be highly variable. As the distinction between cervical intraepithelial neoplasia (CIN) and non-neoplastic conditions guides patient management, women with biopsies overdiagnosed as CIN may receive unnecessary surgical treatment. Benign mimics of CIN (e.g. squamous metaplasia, atrophy, and inflammation-induced atypia) are the biggest source of this variability causing potentially problematic management of patients with false-positive interpretations. Theoretically virtually all CIN are human papillomaviruses (HPV) associated; a novel in situ hybridization (ISH) assay* designed to detect the majority of both low and high risk HPVs known to be causative of CIN lesions could improve the ability of pathologists to accurately identify CIN. Therefore, this study was designed to assess the potential improvement in diagnostic accuracy of cervical biopsy interpretation when using this novel HPV ISH adjunctively with hematoxylin-eosin (H&E) stained slides.

Methods: Fifteen community pathologists evaluated 216 H&E stained cervical specimens for the presence or absence of CIN lesions. Results were compared with an adjudicated reference standard consensus interpretation by three expert gynecopathologists. After a wash-out period of four weeks, slides were randomized and re-evaluated by community pathologists adjunctively with the corresponding HPV ISH slides.

Conclusions: A significant improvement in specificity (p<0.05) was observed when the HPV ISH assay was interpreted adjunctively to H&E. The average diagnostic accuracy for recognizing cases negative for CIN improved from 81.2% to 97.2%. The rate of false-positive interpretations of CIN decreased by two-thirds. These data demonstrate use of HPV ISH as an adjunctly to H&E. The average diagnostic accuracy for recognizing cases negative for CIN improved from 81.2% to 97.2%. The rate of false-positive interpretations of CIN decreased by two-thirds. These data demonstrate use of HPV ISH as an adjunct to H&E provides a significant improvement in diagnostic accuracy with the majority of very common false-positive CIN diagnoses being correctly downgraded to negative for neoplasia.

* not commercially available

AUTOMATED SCREENING OF GYNECOLOGICAL CYTOLOGY: A COMPARISON OF RESULTS

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Objective: To compare the results between conventional gynecological cytology, liquid based cytology (ThinPrep) with manual screening and liquid based cytology (ThinPrep) with automated screening.

Methods: The results of 53,311 cervico-vaginal samples from women in Barcelona, Spain were reviewed. The cases were subdivided into 19,742 conventionally screened samples, 17,596 liquid based, manually screened samples and 15,973 liquid based samples screened with an automated process. We reviewed the cytological diagnoses of 15,973 gynecological cytologies performed from February to December of 2007, using automated screening. In addition, we reviewed the cytological diagnoses of 17,396 cases made from February to December of 2005 using liquid based cytology with manual screening and 19,742 cases performed from February to December of 2002 using conventional cytology. These additional cases did not correspond to the same patients, but were originated from the same districts of the city. We also reviewed the follow-up of these cases to confirm the diagnosis. No ethics review was sought for this project. All of the HSIL cases were biopsied. All cases were reviewed by the same cytotechnologists and cytopathologists. The conventionally and manually screened samples were stained with the usual Papanicolau stain. The Papanicolau staining procedures were followed for the manually screened cases. The stain used for the automated screening was a modified Papanicolau stain for the ThinPrep Imager (Hologic, Inc. Marlborough, MA). All of the liquid based cytologies were processed using the ThinPrepT–3000. The automated screening required an accurate control of times and stainings, in order to achieve optimal working of the Imager and to provide the best results of the automated screening.

Conclusions: Automated screening increases the ASC-US and LSIL detection compared to manual and conventional cytology. In the cases with follow-up automated screening increases the diagnosis of LSIL and HSIL. Automated screening using the ThinPrep Imager increases the number ASC-US and LSIL cases detected, as well as the detection of biopsy confirmed HSIL.
THE ITALIAN ABRUZZO STUDY: LIQUID BASED CYTOLOGY & THINPREP IMAGING SYSTEM VS CONVENTIONAL CYTOLOGY & FOCALPOINT SYSTEM (ARINT STUDY)

Maccallini V1, Angeloni C2, Palazzo F3, Leo C4 and Abruzzo Cervical Cancer Screening Group5

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3 Institute for Research on Population and Social Policy, National Research Council (CNR-IRPPS), Rome, Italy
4 Institute of Clinical Physiology, National Research Council (CNR-IFC), Pisa, Italy - 5 Cervical Cancer Screening Programme, Abruzzo, Italy

Objectives: The Abruzzo Region New Technologies Improvement Project (ARINT) has been initiated to compare liquid based cytology & ThinPrep Imaging System (LBC-TPS) (Hologic, Inc. Marlborough, MA) to conventional cytology & FocalPoint System (CC-FP) (Becton Dickinson, Franklin Lakes, NJ).

Methods: Preparation and computer-assisted screening were centralized: LBC-TPS in Sulmona Hospital and CC-FP in Atri Hospital. The 6 local programmes were equipped with remote review stations. Abruzzo-resident women in the screening age range were included in the programme, as invited or spontaneous participants, 50% with LBC-TPS and 50% with CC-FP. Randomization was done by alternating between the two systems every two months. Clinical data were collected from 180,000 women (90,000 for each arm) over 3 years. All women provided informed consent. Women with ASC-US cytology results or worse were referred for colposcopy and HPV testing.

Conclusions: These results are preliminary and must await confirmation by the final validation. Over 3 years, 117,644 women were recruited: 52,656 CC-FP (44.8%) and 64,988 LBC-TPS (55.2%). Samples from 111,415 women were assessed by computer-assisted cytology. There have been 5,193 ASC+ diagnoses (LBC-TPS: 3,596 vs CC-FP: 1,597). LBC-TPS detected more abnormalities than CC-FP (LBC-TPS: 69.2% vs CC-FP: 30.8%). The PPV for CIN2+ for both systems is equal but LBC-TPS detected more CIN2+ lesions (LBC-TPS: 2 vs CC-FP: 1).

The data confirm that LBC-TPS is associated with a reduction of inadequate specimens. LBC-TPS allowed increased productivity (time saving) and better satisfaction of cytologists, due to improved performance related to ease of use and ergonomics. As in other studies, the results also confirm that LBC-TPS finds more low and high grade cytological diagnoses with good PPV. An important result for cytologists is the reduction of uncertain diagnoses in favour of definite ones.

MULTICYTE™ THINPREP® IMAGING: THE SCOTTISH EXPERIENCE

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Objectives: Computer assisted screening of cervical smears using the ThinPrep® Imaging System (TIS) has shown improved qualitative and quantitative gains in different scenarios but its use has not been described in a well established national screening programme with a low incidence of high grade dyskaryosis.

This is a prospective parallel randomised study, comparing manual screening with image-guided (Dual Review™) screening in order to assess the feasibility of using ThinPrep®MultiCyte™ in the Scottish Cervical Screening Programme (SCSP).

Methods: The study consisted of 169917 ThinPrep® slides, 79366 imaged and 90551 manually screened, between October 2008 and September 2009. Six laboratories from four Health Boards randomly allocated slides to each study arm and imaging was managed within two hub and spoke arrangements. Qualitative data was extracted from the national computer system and quantitative data was recorded manually. Standard laboratory reporting profiles of the SCSP, including sensitivity and specificity of all grades of abnormal smears, positive predictive value (PPV) for high grade abnormalities, false negative rates and screening rates were compared for both study arms. Imager data transfer, transportation and synchronisation of deliveries between sites and imager capacity were also assessed.

Conclusions: Computer assisted screening using the Thinprep® Imaging System in a well established screening programme showed statistically improved productivity without loss of quality. There were statistically significant differences between the insufficient rates, negative rates and low grade abnormal rates in the manual and imager arms. Detection rates for high grade abnormalities and PPV were higher in the imager arm. They showed an upward trend as the trial progressed but did not reach statistical significance. A trend towards statistically significant improved detection of high grade abnormalities should be clarified by extension of the trial. TIS has the potential to maintain screener vigilance in lower prevalence situations such as cervical cytology screening in a vaccinated population. The study findings should be used to inform future screening policy for the SCSP or comparable programme.
A RANDOMIZED TRIAL COMPARING CONVENTIONAL CYTOLOGY TO LIQUID BASED CYTOLOGY WITH COMPUTER-ASSISTANCE: RESULTS OF THE RHINE-SAAR-STUDY

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Objectives: Germany does not have an organized cervical cancer screening program. However, three-year participation rates in its opportunistic program equal those in Great Britain or the Netherlands while cervical cancer incidence is still considerably higher. A recently published study within an organized program did not show significantly increased sensitivity for cervical intraepithelial neoplasia (CIN) with ThinPrep liquid based cytology (TP-LBC) as compared to conventional cytology (CC). Against this background the professional associations of gynaecologists (BVF) of Rhineland-Palatinate and Saarland in Germany conducted a large randomized controlled trial to compare the use of LBC, alone and in combination with computer-assisted ThinPrep imaging technology (TIS), to CC.

Methods: 20,607 women above 19 years attending routine screening at 20 gynaecologic practices in Germany were included. Women were tested with LBC or CC in a direct-to-vial approach between August 2007 and October 2008. All LBC slides were additionally read with TIS. The practices were randomized weekly to use LBC (n=11,331) or CC (n=9,296). The evaluation of smears was only performed by experienced cytotechnicians. All women with cytologic abnormalities (≥Pap III = ASC-H/LSIL/HSIL) were invited for expert colposcopy including biopsy if indicated. Biopsies were reviewed by two independent pathologists. The trial outcome was the detection of histologically confirmed CIN2+ lesions.

The relative sensitivity of LBC versus CC using the CIN2+ cut-off was 2.74 (95% confidence interval [CI] 1.66-4.53). The relative sensitivity of TIS versus CC for CIN2+ was 3.17 (95% CI 1.94-5.19). The positive predictive value (PPV) of LBC and CC for CIN2+ was 48% and 38%, respectively. The PPV of TIS and CC was 44% and 38%. Differences between LBC and CC were smaller in some sensitivity and subgroup analyses; however relative sensitivity of LBC remained significantly increased.

Conclusions: Under the field conditions of an opportunistic screening system LBC without and with TIS compared to CC had a significantly higher sensitivity for the detection of CIN without deterioration of PPVs.

DETECTION OF HUMAN PAPILLOMAVIRUS ASSOCIATED GENOMIC INSTABILITY IN CERVICAL FFPE BIOPSIES

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Objectives: Cervical intraepithelial neoplasia (CIN) induced by infection with high risk (hr) HPV is traditionally diagnosed by histological assessment of cervical biopsies followed by surgical treatment to prevent progression to invasive carcinoma. However, over-treatment, particularly in young and pregnant women, may lead to adverse obstetric outcomes. Therefore, stratification of patients with high risk HPV infections to distinguish transient from carcinogenic infections is highly desirable. As many clinicians prefer histological assessment of biopsy specimens for clinical decisions, the aim of our study was to establish and validate a biopsy protocol for the Abbott Cervical FISH assay, a test originally developed for determination of HPV-induced genomic instability of cervical LBC specimens. The assay uses fluorescent-labeled probes for simultaneous detection of hr HPV and amplification of TERC and MYC, two loci frequently amplified in invasive cervical carcinoma and precancerous lesions.

Methods: A hybridization protocol for formalin-fixed paraffin-embedded specimens (FFPE) was established by using LBC and FFPE blocks prepared from HeLa cells followed by validation for use in our laboratory routine by testing 105 FFPE specimens (21 cases from squamous hyperplasia and/or epithelial inflammation, 20 CIN1, 19 CIN2, 24 CIN3 and 21 invasive squamous carcinomas). Tissue blocks were subsequently cut for H&E (initial diagnosis), FISH, molecular HPV typing, immunohistochemistry (p16, Ki67), and final H&E. Sections were pre-treated for FISH using our standard procedure for FFPE on an automated slide processor (VP2000) after removal of paraffin and the Cervical FISH assay was carried out as described for LBC samples by the manufacturer.

Results: Significantly increasing numbers of cells with amplification of TERC (P<0.001), MYC (P<0.001), and HPV integration (p=0.003) were found with increasing severity of histological diagnosis.

Conclusions: Cervical FISH on FFPE is a suitable and robust method to detect HPV infection and HPV-associated genomic instability providing additional information on the severity of HPV-induced cervical lesions and may be of value for prognostic stratification of patients with high risk HPV infections.
**Risk of Invasive Cancer in Routine Clinical Practice Following a CIN2 or CIN2/3 Biopsy**

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**Objective:** To characterize the short-term risk of invasive cervical cancer at different ages following a biopsy diagnosis of cervical precancer (CIN2, CIN2/3, or CIN3) in routine clinical practice.

**Methods:** Using data from the Kaiser Permanente Northern California (KPNC) Regional Lab, we investigated the cases of cervical cancer diagnosed between January, 2003 - June, 2009, following a biopsy diagnosis of precancer. Inclusion required a CIN2, CIN2/3, or CIN3 biopsy, and at least one subsequent cervical screening test or histologic diagnosis. Follow-up was measured as time from the original biopsy diagnosis to the last evaluation.

**Conclusions:** We identified 2,225 women with CIN2, 1,030 with CIN2/3, and 1,756 with CIN3 with median follow-up of 13.7, 15, and 10.5 months respectively. Four hundred sixty-six women (20.1%) with CIN2, 174 (16.6%) with CIN2/3, and 158 (9.0%) with CIN3 were aged 20-24; 1,759 (79.9%) with CIN2, 856 (83.4%) with CIN2/3, and 1,598 (91.0%) with CIN3 were aged 25-64. None of the 798 women (0.0%, 95% CI = 0.00% to 0.46%) aged 20-24 was diagnosed with invasive cancer in the 12 months following their index biopsy. By comparison, 25 of 4,213 women aged 25-64 (0.59%, 95% CI = 0.38% - 0.87%) with a index cervical precancerous diagnosis were diagnosed with cervical cancer (4/1,759 women with CIN2, 6/856 women with CIN2/3 and 15/1,598 with CIN3 in the ensuing 12 months). The 12-month risk of invasive cervical cancer following a precancerous biopsy for women aged 20-24 was significantly less than it was for women aged 25-64 (p = 0.025, two-sided Fisher’s exact test). In the absence of the ability to demonstrate that cervical cancer screening is beneficial under the age of 25, the onus is on those who continue to recommend this practice to reduce the harms of screening. Based on the data above, we are unable to demonstrate a benefit to the detection of CIN2, CIN2/3 or CIN3 in women age 20-24, in agreement with Sasieni et al.(1) A first step towards more rational management of women under 25 who are found to harbor CIN2 or CIN2/3 would be reevaluation at a one-year interval. This policy would eliminate immediate excisional treatment in 640/798 (80%) of women diagnosed with cervical precancer between ages 20 and 24.

**Current Management of Cervical Pre-Cancer in Low-Resource Settings**

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Cervical cancer is the main female cancer in most developing countries killing approximately 270,000 women worldwide each year. Several strategies have been proposed for early detection of pre-cancerous lesions including conventional Pap smear, low-tech options such as VIA, and highly sensitive methods such as HPV-DNA testing. But independently of the screening method selected, women suspected of harboring pre-cancerous lesions should complete treatment in order to interrupt progression of the disease.

Historically, women with positive screening results were requested to complete diagnostic procedures with colposcopy and biopsy to have a histological diagnosis of the severity of the pre-cancerous epithelial changes, but several experiences from different continents have shown that a precise biopsy-proven diagnosis is needed only in a small percentage of women with suspected invasive cancer or large lesions that make them ineligible for ablative treatment. Fortunately most women with positive screening results have small lesions that can be locally treated using the screen-and-treat approach, facilitating prompt access to definitive treatment.

Cryotherapy is the ablative method more widely used in recent years for treatment of pre-cancer or even In situ carcinoma. The advantages of the method are its simplicity, opening the opportunity for non-physicians to provide treatment; the high acceptability by users and providers; and its low cost. In addition, it does not need electricity or anesthesia and has very low complication rates, with mostly minor side effects such as vaginal discharge or spot bleeding. Among the challenges for implementing the method are assuring adequate gas supply and technical maintenance for the equipment in remote areas. Even though cryotherapy can be used to treat around 80-90% of women with positive screening results, there is still an important number of women that would need to be referred to higher levels of the health care system for additional evaluation or excisional treatment with LEEP or cold-knife cone.

Access to adequate training to get highly-skilled providers for screening and treatment of pre-cancer is still a limitation in many developing countries. Recent experiences from Latin America with the creation of training excellence centers (TEC) have shown that building a team of validated master trainers is extremely important for expanding access to alternative screening and treatment options.
RANDOM BIOPSY DETECTS ADDITIONAL HIGH-GRADE CERVICAL DISEASE IN WOMEN WITH NORMAL COLPOSCOPY

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**Objective:** To demonstrate the diagnostic value of random biopsy/ECC (endocervical curettage) in detecting high-grade cervical disease in women with a normal colposcopy.

**Methods:** The ATHENA trial enrolled 47,208 women and screened 46,887 women using liquid-based cytology and HPV testing with the cobas® 4800 HPV Test that detects HPV16 and 18 individually and 12 other hrHPV types as a pooled result. Colposcopy was performed blinded to all test results in women with ≥ASC-US (atypical squamous cells of undetermined significance; equivocal or borderline) cytology or a positive HPV test result, and in a random subset of women who were cytology/HPV test negative. A single random biopsy was taken if no lesions were visualized and an ECC was performed if the squamocolumnar junction was not seen.

**Conclusions:** Random biopsy in HPV+ women in whom no lesions were visualized, detected approximately 25% additional high-grade lesions over that found by directed biopsy (table).

<table>
<thead>
<tr>
<th>≥CIN2</th>
<th>Total pop≥25 years (n=7,823)</th>
<th>ASC-US ≥21 years (n=1,578)</th>
<th>NILM ≥30 years (n=4,258)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cobas 4800 HPV Test result</td>
<td>Directed, % (95% CI)</td>
<td>Directed &amp; random, % (95% CI)</td>
<td>Directed, % (95% CI)</td>
</tr>
<tr>
<td>HPV16/18+</td>
<td>15.4 (13.9, 17.4)</td>
<td>20.4 (18.5, 22.5)</td>
<td>19.4 (15.5, 24.1)</td>
</tr>
<tr>
<td>12 other hrHPV+</td>
<td>5.7 (5.1, 6.5)</td>
<td>7.2 (6.3, 7.8)</td>
<td>6.5 (4.7, 9.0)</td>
</tr>
<tr>
<td>hrHPV-</td>
<td>0.8 (0.6, 1.2)</td>
<td>1.2 (0.9, 1.5)</td>
<td>0.6 (0.2, 0.9)</td>
</tr>
</tbody>
</table>

| ≥CIN3 | cobas 4800 HPV Test result | Directed, % (95% CI) | Directed & random, % (95% CI) | Directed, % (95% CI) | Directed & random, % (95% CI) |
|-------|-----------------------------|---------------------------|-------------------------|
| HPV16/18+ | 12.0 (10.7, 13.7) | 15.5 (14.0, 17.3) | 12.6 (9.8, 16.1) | 15.9 (12.5, 20.0) | 7.5 (5.8, 9.7) | 9.9 (7.8, 12.3) |
| 12 other hrHPV+ | 3.3 (2.8, 3.9) | 4.0 (3.6, 4.8) | 3.3 (2.0, 5.3) | 4.4 (2.9, 6.5) | 2.1 (1.6, 2.7) | 2.5 (1.9, 3.1) |
| hrHPV- | 0.3 (0.2, 0.5) | 0.5 (0.2, 0.5) | 0.2 (0.1, 0.9) | 0.3 (0.1, 0.9) | 0.2 (0.1, 0.6) | 0.3 (0.2, 0.7) |

NILM (negative for intraepithelial lesions or malignancy; normal)

The prevalence of disease and the absolute increase in disease detected was highest in HPV16/18+ women in all three populations. Our study supports performing a random biopsy(ies) to diagnose high-grade cervical disease in women undergoing colposcopy without visible lesions.

**SS 7-4**

LOOP CONE OR COIN LOOP FOR CIN 1 – AN AGE TAILORED TREATMENT

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**Introduction:** The treatment of CIN 1 (Cervical IntraepithelialNeoplasia 1) is controversial. The question that is unresolved is if CIN 1 is a transient viral infection, without any clinical importance or it is a the first step in a cascade that will lead to CIN 2, CIN 3 and finally to cervical cancer?

The diagnosis of CIN1 is based on colposcopy with a sensitivity of 60 -70%. The pathological diagnosis is also questionable. Cox in the ATLS study found that only 43 % of the biopsy initially diagnosed as CIN 1 were reclassified as CIN 1 , 41% were downgraded to normal and 12% were upgraded to CIN 2-3. (1)

When using LLETZ (Large Loop Excision of Transformation Zone) as a gold standard of CIN 1 diagnosis, Spitzer 1998 (2) and Siegler 2004 (3) found CIN 2-3 in 21% and 20.3% of the cases respectively.

Because CIN 1 in young women is a transient disease the 2006 Consensus guidelines for the management of CIN recommended follow up for 2 years before performing a treatment. (4)

A randomized trial of expectant management versus immediate treatment for Low grade CIN found that CIN2-3 lesions were found in 17.5% of the women initially treated by LLETZ, but only in 4.9% of the women followed up ( 5). The main problem was that during the follow up period between 27-56% of the patients failed to follow up.

Kalliale (6) examined the long term mortality after treatment of CIN and found that among CIN 1 patients the overall mortality, cancer mortality and all disease mortality were statistically significantly higher than among their reference population. They think therefore that we should change the attitude to CIN 1 lesions.

The treatment of CIN 1 lesions can be classified as destructive Cryotherapy, laser ablation or cervical coagulation or excisional – LLETZ procedure. A Cochrane report from 2010 (7) suggests that there is no overwhelmingly superior technique for eradicating CIN. Laser ablation appears to cause more perioperative severe pain, and perhaps more primary and secondary hemorrhage compared to LLETZ.

They mention the ability of LLETZ procedure to diagnose microinvasive disease missed by the colposcopy and cervical biopsy.

The consensus guidelines (4) mention that in CIN1 patients if the colposcopy is unsatisfactory the ablative procedures are unacceptable. They also recommended a diagnostic excisional procedure if the ECC is abnormal or the patient has been previously treated.

The main concern of LLETZ treatment is the risk of pregnancy complications especially premature delivery. A meta-analysis published by Arbyn in 2008 (8) found that LLETZ and ablative treatment with cryotherapy or laser were not associated with a significantly
increased risk of serious adverse pregnancy outcomes and the editorial of BMJ concluded that Large loop excision of the transformation zone was not associated with severe pregnancy outcomes, which is reassuring to clinicians and to women having this treatment. Noehr (9) published a study where it was found that if the depth of the cervical cone removed at LLETZ procedure was less than 15 mm the relative risk of premature delivery was only 0.82.

The ideal treatment for CIN1 should combine an accurate diagnostic procedure with a minimal cervical damage especially in young patients.

**Method:** The aim of our study was to assess the pathological outcomes of women with CIN1 who were treated by Loop Excision of the Transformation Zone (LETZ), and to define the incidence of CIN 2-3 in different age group of patients with CIN1 patients.

We summarized our data of 670 women diagnosed with CIN 1 treated by LLETZ from 2001 till 2010. From 2004 till 2010 patients under 35 years with persistent lesion of CIN1 for more than 12 months until 2007 or more than 24 months after 2007 were treated by a superficial LOOP Coin. In patients older than 35 years with a persistent CIN1 finding a standard LLETZ was performed. The final pathological results, the volume and the height of the cone were compared and the complications of the procedure were recorded in the two groups of patients.

**Results:** Data of 670 cases of CIN1 treated by LETZ found 3 cases of cervical carcinoma, in 124 women (18.5%) CIN 2-3 was diagnosed, in 41.9% CIN1 was found and in 39.4% of the patients the histology was normal. We collected data from 222 women and measured the height of the Loop cone or coin and calculated the volume of the conus. In young women age 18-24 years the average volume and height of the conus were 1.47 cm³ and 0.59 cm, these parameters gradually increased in size to an average volume of 2.76 cm³, and an average height of 0.77 cm in women at age of 35-44 years and an average volume of 2.79 cm³, and an average height of 1.05 cm in women at the age of 45-54 years.

The complications rate was low and included severe hemorrhage in 1.8% of the patients that were treated by repeat coagulations of the cervix.

**Conclusions:**

CIN 2-3 was diagnosed in 18.5% and cervical carcinoma was found in 3 women treated by LLETZ procedure because of CIN 1 lesions.

We think that LLETZ should be performed in CIN 1 cases and to tailor the treatment by a superficial LOOP Coin in young patients diagnosed with CIN 1 is a procedure that combined the advantages of getting a correct diagnosis with the treatment of the neoplasia with the minimal potential risk for future pregnancy complications.

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**ABSTRACTS**

**SS 7-7**

**PHOTODYNAMIC THERAPY (PDT) OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)**

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The study was sponsored by Photocure ASA, Norway.

**Objective:** Invasive treatment of CIN2-3 is associated with increased risk of prenatal complications. Hexaminolevulinate (HAL) PDT works by activating tissue porphyrins using light, offering a selective non-invasive modality that preserves normal tissue.

**Methods:** This was a dose-finding study performed in 92 patients with CIN1-3 using topical administration of methyl aminolevulinate (MAL) 1.6g, HAL 25mg and 100mg solutions applied for 3 and 12 hours. Red light (633nm) was powered by a Ceralas diode laser (Ceramoptec, Bonn) and applied to the cervix by using a cervical light applicator with a total dose of 25-100 J/cm² for 17 minutes. A re-PDT was performed if abnormal cytology at 1 month. Primary end-point was biopsy and Pap-smear at 6 months. HPV testing (Hybrid Capture, Qiagen, Hilden) was also performed.

**Conclusion:** Ninety-two patients (8 CIN1, 41 CIN2, 43 CIN3) were included. A three hour application proved better compared to 12 hours. Light doses of 50-100J/cm² were superior to 25J/cm², but the 50J/cm² showed improved patient satisfaction compared to 100J/cm². Patient response was similar with all drug doses, but patients with CIN1/2 responded better (58%) compared to CIN3 (24%). 83% of CIN2 patients had a complete (CR) or partial response (PR), avoiding patient conization. In CIN1/2 responding patients there was a corresponding 90% HPV response in patients with a baseline infection and 12 month data indicated a sustained effect.

**Table 1**

<table>
<thead>
<tr>
<th>Drug and light regimen</th>
<th>CIN1-2 Lesion CR</th>
<th>CIN1-2 HPV CR</th>
<th>CIN2 Lesion CR + PR</th>
<th>CIN2 HPV CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAL/HAL 3 hrs 50-100J/cm²</td>
<td>58% (14/24)</td>
<td>90% (9/10)</td>
<td>83% (19/23)</td>
<td>83% (10/12)</td>
</tr>
</tbody>
</table>

Patients experienced mild to moderate vaginal discharge and uterine cramping during photoactivation that was reduced by lowering light intensity. A biopsies review at 6 months follow up showed no adverse effects of PDT to normal stroma, documenting the selectivity of the procedure. This explorative study showed promising effects in patients with CIN1/2, but data needs to be confirmed and compared to spontaneous regression (placebo). PDT is a promising tissue preserving treatment modality for patients with CIN and a novel intravaginal combined drug/light system is under clinical development.
Colposcopy is a technique of magnified visualization of the surface epithelium of the lower genital tract (cervix, vagina and vulva) which is used for topographic localization and description of lesions. This technique is included and used within the preventive algorithms of cancer of these localizations, in the second or third step, with subsequent therapeutic and/or follow-up implications.

In most European countries colposcopy is carried out by the gynecologists, as part of their preventive clinical practice or within the frame of a screening program, either a population based or opportunistic.

In 2007 we realized that the quality of the teaching of colposcopy that the Spanish gynecologists were receiving wasn’t well enough. This view was shared by the European Federation of Colposcopy (EFC). For this reason, at a Spanish level an educational effort aimed at the gynecologists was started by the Scientific Societies, in collaboration with the EFC. Presental courses, high quality audiovisual material and websites with up to date contents are all available for those specialists who wish to maintain or improve their professional skills. A survey which is currently underway is trying to assess if all this educational activity has improved the current colposcopic knowledge of our colleagues. The provisional results of this survey will be presented.

As well as this educational effort, a process of establishment of quality controls for the colposcopic practice is currently taking place, specific for each country. This whole effort is overseen and controlled by the EFC. The conclusions of the meeting regarding this subject which will be celebrated in Berlin on April 8th and 9th will be presented.

For too long medicine has adopted the paternalistic attitude of knowing what is best for all patients either from professional body guidelines or from the physician’s own personal preferences for patient behaviors. This era of health care now remunerates providers based on patient outcomes, but more pervasively on satisfaction with the process of health care they received. This requires physicians and health care providers to move to a shared decision making model of health care where patient’s values about the health care outcome is paramount.

It is no longer acceptable to purport to provide education about a health care issue such as cervical cancer prevention when only one perspective is emphasized to effect a pre-specified behavioral outcome. No longer do we require women to get a Pap test before renewing their birth control prescriptions. Women have a choice. Their first choice is characterized as whether to participate in a prevention program. A woman weighs the evidence of how cervical cancer occurs, what her own estimation of risks for cervical cancer are, what the screening processes entail, and her value for each of those. Should she opt for cervical cancer prevention, her second decision delineates which elements of a prevention program she would like to choose: screening and/or vaccination? These decisions, previously never offered to patients due to the directed non-participatory care model provided, now present patients with more information needs.

Decisional support involves providing information to resolve decisional conflict. Decisional conflict can arise from four different areas. Uninformed: decision aids help to clarify what the disease is, how to quantify the woman’s risk in terms she understands, to clarify what the risks and benefits each screening option can provide, and to clarify what the risks of any treatment for detected disease may be. Unclear values: how past experiences have colored the patient’s view of these options, which benefits and which harms matter most to the woman. Unsupported: how to guide deliberation and communication with those she trusts. Uncertainty: how to address the quandary of choosing poorly, what is the potential decisional regret of the missed benefits of the option not chosen.

Our concerns are not with the actual choice the patient makes, but with ensuring that the patient makes an informed decision that reflects her values regarding the benefits and harms of the options presented.
HOW SHOULD THE SENSITIVITY OF COLPOSCOPY BE INCREASED?
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The sensitivity of biopsy of a lesion with colposcopic impression of human papillomavirus (HPV), cervical intraepithelial neoplasia (CIN), or cancer [colposcopically directed biopsy] for CIN 3 or cancer (CIN 3+) was reported as 63.5% in a recent review of 1,383 women with cervical cytology of cancer, HSIL, LSIL, or ASCUS with positive HR-HPV that participated in the Shanxi Province Cervical Cancer Screening Studies (SPOCCS I and II) [Pretorius RG, et al. JLGTD;2011] and 49% in the Gardasil Clinical Trials [Stoler MH, et. al. Int J Cancer;2010]. Options for increasing the sensitivity of colposcopically directed biopsy include improving colposcopy skills, repeating colposcopy at 6-month intervals, more liberal use of loop electrosurgical excision procedure (LEEP) or cervical conization, obtaining ‘random’ biopsies in cervical quadrants with normal colposcopic impressions, and obtaining endocervical curettage (ECC).

Increasing the sensitivity of colposcopically directed biopsy by improving colposcopy skills is difficult because the characteristics suggestive of CIN, that is, acetowhite lesions, color, margin, punctuation, and mosaic pattern are not highly predictive of CIN 3+ [Massad LS et. al. JLGTD;2009]. In addition, relatively thin CIN 3 may not be detected by colposcopy regardless of skill [Yang, B. et. al. Gynecol Oncol;2008]. Repeating colposcopy at 6-month intervals has the drawback that many (34% [Pretorius RG et. al. Am J Obstet Gynecol;2006]) women fail to return for follow-up and a more liberal use of LEEP and cervical conization will likely be associated with an increased risk of subsequent perinatal mortality and other severe adverse pregnancy outcomes. [Arbyn M, et. al. BMJ;2008] We advise ‘random’ biopsies at the squamocolumnar junction in cervical quadrants without visible lesions because 25.7% of the CIN 3+ in our review of the colposcopy experience in SPOCCS I and II was diagnosed by such ‘random’ biopsy, [Pretorius RG et. al. JLGTD;2011] These multiple cervical biopsies should be small (2-3-mm) because the small biopsies are less painful. Though the yield of ECC may be appreciable in an older population with a high risk of CIN 3+ [Pretorius RG et. al. JLGTD;2011], its use in younger women in a screened population is limited. [Gage JC et. al. Am J Obstet Gynecol;2010]

TOWARDS A NEW IFCPC COLPOSCOPY TERMINOLOGY
Jacob Bornstein
for the IFCPC Nomenclature Committee

Objective: During the XIII World Congress of the International Federation for Cervical Pathology and Colposcopy (IFCPC) held in New-Zealand in 2008, the IFCPC established a new Nomenclature Committee.

Methods: The charges of the committee are:
a. To prepare an up-dated, user-friendly colposcopic nomenclature for colposcopists worldwide.
b. To incorporate into the new nomenclature critiques that have been voiced since the 2002 proposal.
c. To add terminology of different loop excision techniques.
d. To consider adding vulvar and vaginal nomenclature.
e. To examine the current nomenclature by an evidence-based medicine approach.

The committee reevaluated previous IFCPC colposcopy terminologies, and related particularly to such aspects as: description of the adequacy of the colposcopic examination, reliability of findings, the differentiation of major and minor colposcopic changes, and the significance of keratosis.

In addition, the committee discussed whether the following concepts should be introduced into the terminology: size of the lesion, the signs of inner border and ridge, location of the lesion in relation to the transformation zone, the performance of random biopsies, rapidity of white epithelial color appearance, Lugol’s staining results.

The introduction of a colposcopy reporting form was considered.

The committee discussed whether the following risk assessment factors should be included in the IFCPC terminology: cause of referral, woman’s age, whether the colposcopy is satisfactory or adequate, HPV 16 status, and HPV vaccination status.

Conclusions
The considerations addressed by the new IFCPC Nomenclature Committee during formal meetings and using a web-based discussion, will be the basis for a discussion with the European Federation of Colposcopy.
Changes in colposcopy terminology: from disease recognition to pattern recognition.

Sideri M.

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Colposcopy plays a central role in cervical cancer screening as it is used to obtain biopsy after an abnormal screening test; the pathology report of the colpo-directed biopsy is the driver of patient management. Recent data have demonstrated that colposcopy is neither highly sensitive in detecting cervical cancer precursors nor highly reproducible. Reproducibility is an important prerequisite of any test used in population screening systems. Colposcopic terminology has been developed to give names the colposcopic patterns; therefore terminology is used to classify the patterns and serves as a common wording system to describe what is seen. Recently the terminology committee of the IFCPC has made a proposal for a change. The reason for the change is the move from a system that describes the colposcopic patterns seen to a system that classifies what is seen into diagnostic categories. Similarly to cytology and pathology where the description of the cell characteristic seen is translated into diagnostic groupings, colposcopic terminology should move from the description of what is seen into the categorization of colposcopic patterns into diagnostic classes. In this way elementary colposcopic features are grouped into four diagnostic categories: normal findings, abnormal findings grade 1, abnormal findings grade 2, suspicion of invasive cancer. In this way the results of the colposcopic exam is translated into synthetic terms usable by the medical community. The four diagnostic categorization system has a value if it fulfils the following requirements: it is reproducible; and it is clinically meaningful.

Reproducibility: the four categories are generated by three subsequent dicothomous splits: 1 - suspicion of invasive cancer (a) versus no suspicion of invasive cancer (b); if the choice is b, normal (a) vs abnormal (b); if the choice is b, abnormal grade 1 versus abnormal grade 2. K values and agreement is strongly influenced by the number of choices: a direct classification into four/five/six categories gives high variations, in comparison with a two choices categorization, where 50% of agreement is obtained by chance. A three steps dicothomous categorization is the pre-requisite to increase reproducibility. Which are the data that show that this categorization is reproducible? The literature showed that normal from abnormal colposcopic findings is a reproducible feature; while the differentiation between grade 1 and grade 2 abnormal colposcopic findings is less reproducible. However present literature is biased by the lack of an uniform categorization of the abnormal findings, as in many reports the categorization system used was an histological interpretation of the colposcopic findings; for example americans classify colpo findings into normal, metaplasia, low grade, high grade and suspicion of cancer. This type of classification is highly subjective as it adds to the pure classification of what is seen into five classes, the subjective interpretation of the findings into the hypothetically result of the biopsy done in that area; in this way, the classification is trying to predict histology and it utilizes pathologic terms adding another subjective step to an already subjective interpretation.

The clinical meaning: what are the expectations of the colposcopic examination? At present colposcopy is expected to predict the worst area on the cervix where to take a biopsy; and the biopsy result is used to manage patients; so, colposcopic diagnosis is not included into the management algorithm. In other words, colposcopic terminology and classification has little to do with the clinical management of the patient. Apart from the fact that recent papers have questioned the sensitivity of colposcopic directed biopsy in the detection of cervical pre-cancers, the information that colposcopy gives for patient management extends far beyond the selection of the place to put a biopsy; it gives information on the size and topography of the transformation zone as well as the morphologic features of it; all these aspects are integrated with the clinical history of the patient, cytology, virological and molecular data, and eventually with the pathology results of biopsies to make the decision on the clinical management of the patient. The morphological features observed in colposcopy is one of the two colposcopic elements that drive patient management: it adds to the risk stratification system based upon cytology and HPV status an added value as an independent risk stratifier; the biopsy result is only an additional output of the colposcopic examination, but not the most relevant. On the contrary a common view on colposcopy is that it can predict histology: the classification of colposcopic findings into normal, metaplasia, low grade, high grade and suspicion of cancer clearly shows a misunderstanding in the optimal use of colposcopy.

In conclusion the present change in terminology has the aim to change the meaning of the colposcopic examination, from an instrument to take biopsies to an instrument for patient management.
**FIRST LOOK AT EFFECTIVENESS DATA FROM A LONG-TERM EXTENSION STUDY OF GARDASIL IN ADOLESCENTS**

Daron Ferris, on behalf of the Protocol 018 investigators

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**Objectives:** We describe the first interim effectiveness data for a long-term immunogenicity, safety, and effectiveness study of GARDASIL among adolescents.

**Methods:** In the base study, 1,781 sexually naïve boys and girls were assigned (2:1) to GARDASIL or saline placebo at day 1, months 2 and 6. At the end of the base study (month 30), the placebo group received GARDASIL following the same regimen. Those vaccinated with GARDASIL in the base study are the early vaccination group (EVG). Those vaccinated with GARDASIL during months 30-36 are the catch-up vaccination group (CVG). Subjects in the CVG were not tested for HPV infection prior to receiving GARDASIL. As this extension study does not have a placebo arm, effectiveness was assessed by calculating the incidence of the primary endpoints (HPV6/11/16/18 persistent infection or related disease) and comparing these rates with those from previous phase 3 studies in men and women aged 16-26. The median follow-up time for effectiveness was 1.8 years in both the EVG and CVG. The safety objective is to describe the incidence of deaths and serious adverse experiences (SAE).

**Conclusions:** For each gender, the age-specific distribution of anti-HPV 6, 11, 16, and 18 responses at 4 weeks post dose-3 of GARDASIL were comparable in the EVG and CVG, demonstrating continued persistence through 6 years post-vaccination. Rates of acquisition of new sexual partners and common STIs such as Chlamydia and gonorrhea were similar to previous phase 3 studies in men and women, indicating the subjects were sexually active and at risk for HPV. In females, there were no cases of disease or persistent infection related to any of the four vaccine HPV types in those vaccinated 6 years previously (EVG, Intention-to-treat analysis). In the Intention-to-treat population of the CVG, there were 3 cases of HPV18 persistent infection (all 3 were sexually active prior to vaccination, their pre-vaccination PCR status was unknown, and the observed onset of HPV18 persistent infection was ~2 years after sexual debut). There were no cases of HPV6/11/16/18 persistent infection among males. As of the data cut-off date, there were no data available to assess disease endpoints in males. One SAE was reported, a fatal road traffic accident. For any HPV vaccine, long-term follow-up of disease endpoints is required to establish the duration of protection. This study of GARDASIL provides the first long-term effectiveness data among males and females vaccinated during adolescence.

**LONG-TERM FOLLOW-UP (LTFU) STUDY OF HPV-TYPE-SPECIFIC DISEASE IN PREVIOUSLY GARDASIL™ VACCINATED WOMEN**

Joakim Dillner for the HPV Vaccine Nordic Follow-Up Team

**Background:** The GARDASIL™ LTFU study is an ongoing extension of a pivotal randomized, placebo-controlled, double-blind, phase III study FUTURE II (Protocol 015) that investigated the safety, immunogenicity, and effectiveness of quadrivalent Human Papillomavirus (qHPV) vaccine on the incidence of HPV 16/18-related CIN 2+ in 16-to 23-year old women. The present report includes follow-up to average 6 years after vaccination, with follow-up of up to 7 years in some subjects.

**Methods:** Follow-up of subjects is done through registry-based follow-up for effectiveness as well as safety. Effectiveness and safety analyses occur approximately 2 years following completion of P015 and approximately every 2 years thereafter for 10 years. The current report represents the first of these analyses. Cohort 1 included approximately 2,700 subjects who received qHPV vaccine at the start of P015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and qHPV vaccine prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN 2+ was estimated by calculating the expected incidence of CIN 2+ in an unvaccinated cohort using historical registry data. For analysis of long-term HPV type replacement or cross protection, the incidence of HPV-associated disease by HPV type was analysed among women who had been generally HPV Naïve (GHN) at vaccination.

**Results:** There were no cases of CIN 2+ observed in the GHN population irrespective of HPV type. There were seven (7) cases of CIN observed with follow-up time of 1,074.6 person-years (PY) regardless of HPV type in the GHN population, incidence rate for this endpoint was 0.7 (95% CI: 0.3, 1.3) per 100 PY at risk. The incidence rates for CIN related to any of the 10 non-vaccine HPV types and not related to any of the 14 assay-identified HPV types were 0.4 (95% CI: 0.1, 1.0) and 0.2 (95% CI: 0.0, 0.7) per 100 PY, respectively. By comparison, the incidence rates seen during the Phase III studies were 2.4 per 100 PY (95% CI 2.4, 2.6) irrespective of HPV type, 2.0 (95% CI 1.8, 2.2) for the 10 non-vaccine HPV types and 0.5 (95% CI 0.4, 0.6) not related to any of the 14 HPV types assayed.

**Conclusions:** Although based on limited number of observations, the data are suggestive of an absence of any increase in disease associated with non-vaccine HPV type (type replacement). The incidences of HPV-associated disease by HPV type will continue to be assessed and further analyses will be performed at two-year intervals.
DOES THE HPV-16/18 AS04-ADJUVANTED VACCINE BENEFIT WOMEN WITH CERVICAL DISEASE?

Garland S* on behalf of the HPV PATRICIA Study Group

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Objectives: The AS04-adjuvanted human papillomavirus (HPV)-16/18 vaccine shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We investigated whether women who underwent subsequent cervical excision during the PATRICIA (NCT00122681) trial benefited from reduced incidence of new genital disease following vaccination in the end-of-study analysis.

Methods: Women aged 15–25 years, irrespective of baseline HPV/DNA-status, serostatus, or cytology, were randomised to receive HPV-16/18 vaccine (n=9,319) or hepatitis A vaccine (control; n=9,325) at Months 0, 1 and 6. Cervical samples were collected every 6 months for HPV DNA typing; gynaecological and cytological examinations (with histological sample when necessary) were performed annually. Incidence rates (IR; women with ≥1 event ≥60 days post-treatment per 100 person-years follow-up) and VE with 95% confidence intervals are reported for women who underwent surgical therapy (loop electrosurgical excision procedure [LEEP], cone, knife or laser) in the total vaccinated cohort (women receiving ≥1 vaccine dose, regardless of baseline characteristics). All analyses are irrespective of HPV type.

Conclusions: 190 women in the HPV-16/18 vaccine group and 264 in the control group underwent surgical therapy. The number with subsequent CIN2+ (CIN2, CIN3, adenocarcinoma in situ, or invasive cervical cancer) ≥60 days after treatment of a first cervical lesion was 1 in the HPV-16/18 vaccine group and 9 in the control group. The IR of CIN2+ post-surgery was 0.24 (0.01–1.32) and 2.01 (0.92–3.81) for the HPV-16/18 vaccine and control groups, respectively. VE against CIN2+ was 88.2% (14.8–99.7). The number of women with CIN1+ (CIN1 or CIN2+) ≥60 days after treatment was 12 in the HPV-16/18 vaccine group and 22 in the control group. The IR of CIN1+ post-surgical therapy was 2.91 (1.50–5.08) for the HPV-16/18 vaccine group and 5.07 (3.18–7.68) for the control group. VE against CIN1+ was 42.6% (-21.1–74.1). Women who undergo surgical therapy after vaccination with the HPV-16/18 vaccine can continue to benefit due to reduction in the risk of developing further or recurrent CIN1+ or CIN2+ lesions.

IMMUNE RESPONSE TO THE HPV-16/18 AS04-ADJUVANTED VACCINE ADMINISTERED AS A 2-DOSE OR 3-DOSE SCHEDULE 2-YEARS AFTER VACCINATION

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Background and aims: The HPV-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Biologicals) is highly immunogenic using its licensed 3-dose (3D) vaccination schedule. This study (NCT00541970/110659) evaluated 2-dose (2D) schedules using the licensed vaccine formulation (20 μg of HPV-16 and 18; 20/20) or an alternative formulation (40 μg of each antigen; 40/40), compared with the standard 3D schedule. We present safety and immunogenicity follow-up to Month (M) 24.

Methods: Healthy females (age-stratified: 9–14, 15–19, 20–25y) were randomised to receive HPV-16/18 vaccine (20/20) at M0,1,6 (n=239), 40/40 at M0,6 (n=241), 40/40 at M0,2 (n=240) or 20/20 at M0,6 (n=240). HPV-16/18 antibody levels and vaccine safety were assessed throughout the study 2 years after the first dose. Non-inferiority of the antibody response was demonstrated if the UL of the 95% CI for the GMT ratio between the 3D and 2D schedules was < 2.

Results: At Month 24, all subjects seronegative at baseline were seropositive for both antigens; GMTs were substantially higher than natural infection titers in all groups (see table).

<table>
<thead>
<tr>
<th>Dose/schedule</th>
<th>Month 24 GMTs (95% CI)*</th>
<th>9–25y</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/20 M0,1,6</td>
<td>2390 (2007–2847)</td>
<td>852 (721–1007)</td>
</tr>
<tr>
<td>40/40 M0,6</td>
<td>1776 (1560–2021)</td>
<td>796 (680–932)</td>
</tr>
<tr>
<td>40/40 M0,2</td>
<td>806 (701–926)</td>
<td>332 (284–387)</td>
</tr>
<tr>
<td>20/20 M0,6</td>
<td>1326 (1168–1506)</td>
<td>684 (591–791)</td>
</tr>
</tbody>
</table>

*El.U/ml

At Month 24, 2 doses of HPV-16/18 vaccine (20/20 M0,6) in girls 9–14y were non inferior to 3 doses of HPV-16/18 vaccine (20/20 μg M0,1,6) in women 15–25y, with corresponding GMTs (95% CI) of 1702 (1416–2045) vs 1865 (1505–2311) for HPV-16 and 702 (563–876) vs 728 (588–900) for HPV-18. The kinetics of antibody response in the two groups were comparable. The vaccine had a clinically acceptable safety profile in all groups up to Month 24.

Conclusions: 2 doses of HPV-16/18 vaccine (20/20 M0,6) in adolescents 9–14y were non inferior to 3 doses of HPV-16/18 vaccine in subjects 15–25y.
THE IMPACT OF QUADRIVALENT HPV VACCINE ON EXTERNAL GENITAL BIOPSIES AND SURGICAL/NON-SURGICAL PROCEDURES IN MEN

Anna Giuliano, for The Male Quadrivalent HPV Vaccine Efficacy Trial Team

Background: Previous data have demonstrated the efficacy of the quadrivalent HPV vaccine (qHPV) against external genital lesions (EGL [perianal/perineal/penile intraepithelial neoplasia and condyloma]) in men aged 16-26. In this analysis we examine the impact of the vaccine on the incidence of HPV related biopsy and surgical and non-surgical procedures.

Methods: Data are from male subjects aged 16-26 from Protocol 020 who were randomized to receive vaccine or placebo at enrollment, month 2, and month 6. Subjects underwent detailed anogenital exams as well as sampling from the penis, scrotum, perineal/perianal and anal canal (men having sex with men only) at enrollment, month 7 and at 6-month intervals afterwards. Analyses were performed in all subjects (ITT), and in a population seronegative and PCR negative at enrollment to HPV 6, 11, 16 and 18, who were PCR-negative at enrollment to HPV 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, who received at least one dose of study material, and who had follow-up after Day 1 (naive). Procedures in these analyses include all therapies that could potentially be associated with HPV-related EGL (i.e., surgical therapies included procedures such as surgical excision, laser ablation, cauterrization, coagulation, and cryotherapy, and nonsurgical therapies included topical treatments, including chemical ablation). In addition, investigators were instructed to collect biopsies from any lesion that could possibly, probably, or definitively be associated with HPV or whose relationship to HPV could not be determined, to increase sensitivity of EGL detection.

Results: The incidence of external genital lesion biopsy in the naive population was reduced by 54.2% (95% CI: 28.3, 71.4) in subjects who received qHPV vaccine compared to subjects who received placebo. The overall reduction in surgical and non-surgical procedures in those who received vaccine compared to those who received placebo was 44.7% (95% CI: 18.4, 67.1). Percent reductions in biopsies and surgical/non-surgical procedures were also statistically significant in the ITT population (45.7% [95% CI: 29.0, 58.7] and 38.1% [95% CI: 19.4, 52.6], respectively).

Conclusions: These results indicate a reduction in the incidence of EGL-related biopsies and surgical/non-surgical procedures in subjects vaccinated with qHPV vaccine.

DISEASE-SPECIFIC ADVERSE EVENTS FOLLOWING THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE (qHPVV): ANOTHER SPONTANEOUS REPORTING BIAS?

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Objectives: Because they replicate in vaccines, live vaccines, can cause disease-specific adverse events following immunization (AEFIs), indistinguishable from the symptoms of the disease for which the vaccine is being administered, e.g. measles-like rash following measles vaccination. Non-live vaccines, which do not replicate in vaccinees, are not likely to cause disease-specific AEFIs. Since 2007 HPV immunization is recommended in France for 14-23 y-o-a women to prevent HPV-related gynaecologic diseases. We assess whether safety signals could be generated by a not yet described psychological bias in spontaneous reporting of disease-specific AEFIs with non-live vaccines including qHPVV.

Methods: In France, between January 2000 and June 2010, 33,275 AEFIs with 14 vaccines, spontaneously reported to Sanofi Pasteur MSD and coded according to MedDRA terms, were included. Vaccine failures were excluded. 22 vaccine/AEFI pairs of interest were selected a priori, e.g. qHPVV-reproductive system disorders, tetanus vaccine-trismus. The proportion of a given reported disease-specific AEFI associated with the vaccine administered to prevent that disease was compared to the proportion of this symptom following all studied vaccines. We used the Reporting Odds Ratio methodology to generate safety signals by assessing the disproportionality of spontaneous reporting in databases. qHPVV results were adjusted for age and sex.

Conclusions: A statistically disproportionate reporting of disease-specific AEFIs was found for almost all the pairs tested (16/22). The most spectacular one was the association between gynaecological symptoms and the qHPVV [OR = 13.3 (95%CI 5.0-49.8)]. The strength of the association could be explained by 1) a strong Weber effect, 2) women report more AEFIs compared to men 3) numerous women were vaccinated during a gynaecological visit making them more likely to attribute gynaecological symptoms to the qHPVV. Considering that the selected AEFIs did not fulfil the WHO causality assessment criteria, including biological plausibility, we suggest that the “disease-specific adverse events following non-live vaccines” represent a new unidentified bias of AEFIs spontaneous reporting. This placebo side effect and/or nocebo phenomenon could generate false safety signals and disrupt immunization programs.
SURVEILLANCE OF CONGENITAL ANOMALIES IN BABIES BORN TO WOMEN EXPOSED TO GARDASIL® DURING PREGNANCY

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Objectives: To evaluate the risk of congenital anomalies in infants born to mothers with inadvertent exposure to HPV vaccination (GARDASIL®) during pregnancy.

Methods: In the process of monitoring the impact of HPV vaccination at a population level (the VIP-study) in four Nordic countries (Denmark, Iceland, Norway and Sweden), we will identify women from these four countries who have been inadvertently exposed to GARDASIL® during pregnancy (up to 30 days before estimated day of conception or any time during pregnancy) and who have given birth to a live born child. Among infants born to these women, we will establish whether there are any cases of congenital anomalies (identified at birth or during the first year of life). This will be achieved by using a variety of population-based registries in the respective Nordic country. Standardized Incidence Ratio (SIR) of congenital anomalies in the women with pregnancy exposure to GARDASIL® vs. the general population will be calculated and adjusted for maternal age. Furthermore, medical charts of the mother and the child will be identified and retrieved from the relevant hospitals departments. An independent Teratology Panel, consisting of one teratologist/pediatrician from each country participating in the VIP-study, will review these congenital anomalies by means of the medical charts. Based on their professional experience the panel will determine if the congenital anomaly is likely to be related to the exposure to GARDASIL®. The Teratology Panel meets once every year and the first meeting took place in 2010. The review process is scheduled to continue at least until 2013.

Conclusions: At the first Teratology Panel review meeting 38 women were identified with a suspected pregnancy exposure to GARDASIL®, one mother gave birth to an infant with a small atrial septum defect. This single congenital anomaly case was reviewed by Teratology Panel, and they concluded that it was "probably not related to vaccination". The calculated SIR was 2.1, 95% confidence interval 0.1–61.7. The SIR was based on the observed one case versus an expected 0.48 case; and the 95% confidence interval was wide and overlapping with 1.0.

POST-LICENSE SAFETY STUDY OF QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE AMONG 189,629 FEMALES

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Objectives: Following licensure of the quadrivalent human papillomavirus vaccine (qHPV) in females in the United States, an observational database study was conducted in two large managed care organizations to assess qHPV safety under routine clinical use. The study evaluated: 1) general safety (post-vaccination emergency room (ER) visits and hospitalizations); 2) new onset of 16 pre-specified autoimmune conditions among all females, and 3) pregnancy outcomes (congenital anomalies, miscarriages) among females with inadvertent qHPV vaccination during pregnancy.

Methods: The study provided safety data on all females (n=189,629) who received at least one dose of qHPV at Kaiser Permanente (KP) Northern and Southern California between August 2006 and March 2008. General safety outcomes were evaluated by comparing incidence of ER or hospital electronic diagnosis codes (grouped into clinically meaningful categories) shortly after vaccination (Days 1-60, 1-14, Day 0) to incidence in a post-vaccination self-comparison period, using conditional logistic regression. Medical records of potential new onset cases of autoimmune conditions after qHPV and pregnancy outcomes (among 2,678 women who received qHPV during pregnancy) were reviewed by expert committees blinded to vaccination status to confirm diagnosis. Incidence of new onset autoimmune conditions was compared to incidence in an unvaccinated KP population. An independent external Safety Review Committee (SRC) evaluated results for potential safety signals.

Conclusions: With the exception of syncope on the day of vaccination (OR 6.0, 95% CI 3.9-9.2) and possibly cellulitis within 14 days of vaccination (OR 1.6, 95% CI 1.2-2.3) (some of which may be misdiagnosed injection site reactions), no safety signals were detected for any health event resulting in an ER visit or hospitalization within 60 days of each vaccination with qHPV. No safety signals associated with pre-specified autoimmune conditions or pregnancy outcomes were identified.
**A Registry-Based Long-Term Follow-Up (LTFU) Study of the Quadrivalent HPV (qHPV) Vaccine in Four Nordic Countries**

Mari Nygård for the HPV Vaccine Nordic Follow-Up Team

**Background:** The LTFU study is an ongoing extension of a randomized, placebo-controlled, double-blind, 4-year clinical trial, FUTURE II, to investigate the safety, immunogenicity, and effectiveness of qHPV vaccine on the incidence of HPV 16/18-related CIN 2+ in young women.

**Methods:** A total of 5498 Danish, Icelandic, Norwegian and Swedish FUTURE II participants were randomized to receive either qHPV vaccine or placebo. Of these, 4847 received at least one dose either start or end of FUTURE II. 96% of participants consented for long term follow-up for Pap tests, cervical, vaginal/vulvar biopsies or definitive therapy procedures by searching the respective, population-based registries. The first search covered the time period from the subject's last FUTURE II visit through 01-Mar-2009. All biopsies and definitive therapy samples were identified from the national biobanks and collected for study purpose. Each country was responsible for obtaining the formalin fixed paraffin embedded (FFPE) tissue blocks, H&E stained slides, and pathology reports from the local laboratories and routing them to a central group, the Nordic Coordinating Center (NCC). The NCC was responsible for sample management, routing blocks for HPV PCR testing and the endpoint adjudication process through the Nordic Pathology Panel (NPP). The LTFU study used the same central laboratories for sample processing and PCR testing and similar adjudication procedures as established in FUTURE II.

**Results:** As of March 1st, 2009, a total of 3,601 Pap screening visits and 589 colposcopy/definite therapy procedures for subjects were identified in the registries. Of the latter, 843 FFPE tissue blocks for 345 cervical/vulvar/vaginal biopsies, 184 endocervical curettage, and 38 conisation procedures were collected, tested for HPV types and completely adjudicated by the NPP. Consensus diagnoses were achieved for different morphology diagnoses (CIN 1-3, AIS, etc) following 2, 3, 4, and 5 NPP reviews, respectively.

Statistical analysis is ongoing and consensus pathologic diagnoses by HPV types and vaccination status will be presented.

**Conclusions:** Long-term follow-up is feasible by using population-based health registries and biobanks with minimal losses to follow-up.

**SAFETY OF A PROPHYLACTIC QUADRIVALENT HUMAN PAPILLOMAVIRUS (TYPES 6, 11, 16, AND 18) (qHPV) VACCINE IN CHINA: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL**

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**Background:** We describe the safety of qHPV vaccine from an MSD-sponsored blinded clinical efficacy study in China.

**Methods:** 3,006 Chinese women subjects aged 20 to 45 years with no history of genital warts or cervical disease were enrolled into a multicentre, randomized, placebo-controlled, double-blind study, stratified by age with 1,855 subjects enrolled in the 20 to 26 years of age group and 1,151 subjects in the 27 to 45 years of age group. Participants received qHPV vaccine or placebo at Day 1, Month 2 and Month 6. The subjects were followed for all adverse experiences (AEs) using Vaccination Report Card (VRC) daily for 14 days after each vaccination. Subjects were solicited for any gynecological health concerns and any serious adverse experiences (SAEs) that have occurred through Day 1 to Month 7. The safety data are blinded as to vaccination group because the study continues.

**Results:** 2,997 subjects received at least 1 dose of qHPV vaccine or placebo. During 14 days following any dose of qHPV vaccine or placebo administration, there were 1728 (57.7%) subjects with one or more clinical AEs, 955 (31.9%) subjects with injection-site AEs, and 1435 (47.9%) subjects with systemic AEs. All injection-site AEs were considered vaccine-related. The incidence rate of injection-site AEs in 20-26 years age group is higher than in 27-45 years age group (34.9% v 26.1%) however, the AE pattern is similar. The intensities of the AEs were mild or moderate in the majority of study subjects of all ages. One subject experienced a vaccine-related serious AE, (name it). There were no deaths and no AE led to discontinuation.

**Conclusion:** In 20-45 years Chinese women, the qHPV vaccine was generally safe and well tolerated.
Introduction: Since 2008, as part of the national immunization program, Dutch teenage girls are offered HPV vaccination to reduce the burden of cervical cancer. Next to routine HPV vaccination of 12-year-old girls a catch-up program was once-only introduced covering girls from 13 to 16 years of age. Vaccine coverage reached approximately 50%, which is low compared to other vaccinations in the Netherlands. Therefore, a large number of girls are still at risk of acquiring an HPV16 or -18 infection later on in life, although the risk might be significantly reduced by herd protection. The aim of this study is to design a dynamic transmission framework to model HPV transmission in the population in order to predict epidemiologic and economic consequences of population based vaccination programs. Here we report on the dynamic transmission model.

Method: We designed a dynamic model to simulate the transmission of the five most prevalent oncogenic HPV-types individually (i.e. 16, 18, 31, 45, 52), other oncogenic HPV-types were grouped into two different groups (i.e. high risk and low risk). The dynamic transmission model framework, e.g. the heterosexual contact matrix and the partner change rate, was parameterized through two large-surveys among 12 - 70 year old Dutch citizens. In the model the population was stratified by gender, age, and sexual activity (in particular, core and non-core groups). Age-specific HPV prevalence observed in the Netherlands and cervical cancer incidence and mortality data were used to calibrate our model assuming a steady state.

Conclusion: Based on the abovementioned data regarding sexual behavior we were able to simulate the transmission of HPV and to simulate the progression from HPV infection to cervical cancer. Our model fitted HPV-prevalence, cervical cancer incidence and mortality quite well. Further work will be directed to simulate the epidemiologic and economic consequences of different HPV vaccination strategies.
SS 10-1

HOW TO EVALUATE EMERGING TECHNOLOGIES IN CERVICAL CANCER SCREENING

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HPV DNA testing for primary screening has been evaluated through randomised controlled trials (RCTs) involving at least two screening rounds. Although a reduction of the occurrence of new invasive cancers has been directly documented, the main studied endpoint was the occurrence of high-grade CIN (in particular CIN3) at the second round. A reduction of hg-CIN at this time in the HPV-screened vs. the cytology-screened group was considered a sufficient surrogate as it showed earlier diagnosis of persistent hg-CIN. Comparing the HPV-screened vs. cytology-screened detection ratio at round 2 in RCTs that used different management of HPV positive women is also a reliable way of comparing such policies. Comparing the study groups as for the cumulative incidence of hg-CIN over the two rounds also allows evaluating overdiagnosis of regressive lesions.

Three types of new technologies are mainly relevant in cervical cancer screening: (a) new HPV DNA tests, (b) new tests different from HPV DNA to be used as primary screening tests (e.g. mRNA of HPV oncogenes, p16-INK4A overexpression) and (c) tests to triage HPV positive women.

As longitudinal studies have already been conducted on HPV DNA for primary screening, for point (a) cross-sectional studies on accuracy for hg-CIN, are sufficient. These should compare the new test to a validated HPV DNA test in unselected healthy women by double testing and have a complete assessment of women positive to either test.

For point (b) the duration of the low risk period after a negative test is frequently unknown. This is essential to define screening intervals. Therefore, in addition, longitudinal studies on the occurrence of new high-grade CIN in test-negative and test-positive women are needed.

For point (c) both studies on cross sectional accuracy and on “longitudinal” accuracy are needed. The former are relevant for the decision to refer HPV positive women for colposcopy, the latter for choosing the interval of follow-up. Studies should be conducted among unselected HPV-positive women, possibly with complete assessment of all of them. RCTs comparing women managed according to different protocols represent the ideal final validation.

SS 10-2

THE VALUE OF SELF-SAMPLING

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Non- or infrequent attendance is one of the main limitations to the success of the cervical screening programme. Targeting non-attendees, representing almost 25% of invited women in the Netherlands, is important, because these women have an increased risk of cervical cancer. In recent years, several efforts have been made to evaluate whether self-collected (cervico-)vaginal material could serve as a good alternative for physician-collected cervical scrapes. Self-sampling may improve screening attendance in developed countries and facilitate access to cervical screening in developing regions.

Recently, we conducted two large studies (PROHTECT) evaluating offering self-sampling using two devices (lavage- and brush-based, respectively) for collecting (cervico-)vaginal material for high-risk human papillomavirus (hrHPV) testing to women not attending regular cytological screening (Gök et al, 2010; 2011). We found that self-sampling is feasible and effective. Response rate to self-sampling was approximately 30%, which was significantly increased compared to a second recall for conventional cytology. The concordance between hrHPV detection in self-samples and corresponding physician-taken cervical scrape samples was very good, particularly among women with CIN2+. Adherence of hrHPV-positive self-sampling women to cytology triage at the general practitioner level, and of those with abnormal cytology to colposcopy referral was high. The CIN2+, CIN3+, and carcinoma yields were 1.4%, 1.0%, and 0.1%, respectively, in self-sampling responders.

Altogether, offering self-sampling for hrHPV testing to non-attendees significantly increases the attendance to the screening program, yields hrHPV test results that are in very good concordance with those of physician-taken scrapes, and is effective in detecting CIN2+/CIN3+. Further improvement of the self-sampling approach may be expected by molecular triage testing directly on self-sampled specimens as alternative to cytology-based follow-up at the general practitioner level. Testing of hrHPV positive self-samples with a methylation marker panel (i.e., for CADM1 and MAL genes) is a feasible approach which currently is under evaluation in a prospective randomized trial.
Prevalence of potentially oncogenic HPV infections as well as mild or borderline cytological abnormalities and of cervical precancerous lesions is generally very high within the youngest targeted age groups of cervical cancer screening programmes. Burden of intensified screenings, triage, and of referrals to further investigations and pre-cancer treatments resulting in conventional cytology-based screening is already very high and the screening programmes should seek strategies to reduce the burden. In case of HPV testing the burden among young women would become unacceptably high, due to the very high prevalence of infections and a high sensitivity of the test to recognise precursor lesions also in a case that the lesion wouldn’t progress. Primary HPV testing in older women, 35 or 40 years or more, may be more feasible than testing younger women. This is partially related to lower prevalence of HPV-positive women at this age, a lower prevalence of pre-cancer lesions in general, and a lower probability of diagnosing a non-progressive lesion. These same aspects may be relevant also for considering triaging of borderline cytological abnormalities with a standard HPV-DNA test. When introducing HPV testing, it is therefore important to consider carefully cost-effectiveness and quality-of-life issues as to which age groups to target the screening programme; and which tests and intervals to use, specific for age groups. Simultaneously, it is essential to reduce the use of any screening services outside the programme; e.g. among women too young for a population-based programme. Otherwise the lifetime burden of screening tests and precursor lesions would increase compared with cytology screening or remain at a high level. Long-term developments in other strategies to control for HPV-related diseases should be taken into account here, too. In older women, what about continuing the programme if there has been a positive HPV test result (or an abnormal cytological finding)? HPV-testing should in principle provide protection over a longer interval than cytological screening, but information up to cervical cancer prevention for testing rather old female population is not available yet.

Background: Anal cancer rates are rising and the high-grade anal intraepithelial neoplasia (AIN2+) is the precursor lesion. Screening for AIN2+ utilizes anal cytology reported in accordance with the Bethesda classification and those with abnormal cytology are referred for high-resolution anoscopy (HRA) while those with benign cytology are re-screened in 1-2 years. As in the cervix, most anal cancer is caused by high-risk (HR) HPV subtypes. Detection of HR HPV by Hybrid Capture 2 (HC2) assay (Qiagen) has augmented cervical cytology. HC2 is not FDA approved for use in the anus. We endeavored to determine if HC2 could be beneficial in anal dysplasia screening.

Methods: We performed a prospective study on patients referred to a surgical practice (SG) for anal dysplasia screening. All patients had two specimens collected at their first visit: a swab for liquid-based cytology (ThinPrep medium, Hologic) and then they were randomized to a swab or brush for HC2 placed into Specimen Transport Medium (STM, Qiagen). After the cytology sample was obtained, HC2 was performed on residual cells. HRA was performed on all patients at a second visit after the swab or brush was used to resample for HC2. Biopsies were taken from lesions suspicious for AIN2+ and pathology was scored based on the highest grade of dysplasia found.

Results: 290 male and 8 female patients enrolled, 134 (44%) were HIV-positive and 103 (36%) had AIN2+. HC2 identified HR HPV in 210 (70%). Nine patients with AIN2+ were HC2 negative. The sensitivity, specificity, NPV and PPV for cytology alone was 77%, 52%, 80% and 48% respectively. For HC2 alone the sensitivity, specificity, NPV and PPV were 91%, 40%, 89% and 46%, respectively. AIN2+ was found in 24 patients with benign cytology (20%), and in 31 patients with ASCUS (35%). When HC2 was combined with cytology so that only ASCUS HC2+ patients had HRA, than sensitivity, specificity, NPV and PPV were 93%, 40%, 91% and 46%. The increased sensitivity over cytology alone is statistically significant (p=0.0011). There was no difference in HC2 HR positivity between swab and brush.

Conclusion: HC2 testing for HR HPV demonstrated improved sensitivity over cytology and may have utility in screening for AIN2+ especially in those with ASCUS, benign or non-diagnostic cytology. STM is the ideal medium. Further study is warranted.
Objective: In Sweden, the last invitation to organised cervical screening is issued to women at age 60. Most advanced cancers are detected in women not having had Pap smears according to the recommendations and in women above the age of 661. We investigated whether a Pap smear after age 61 is beneficial for women aged 66 to 80 years, in terms of reduced risk of invasive cervical cancer.

Methods: All cervical cancer cases ages 66 to 80 in the Swedish nation-wide audit, and their age matched controls, all resident in counties having databases with long term screening history (27% of the cases, 30% of the controls). Screening history was classified by whether a normal Pap smear was taken between age 55 and 61 or not, and if a normal Pap smear taken after age 61, or not, and whether this smear was taken within five or ten years before diagnosis of invasive cervical cancer. Odds ratios (OR) of invasive cervical cancer, depending on screening history were calculated, together with 95% confidence intervals (CI).

Results: In women who had no Pap smear after the age of 61 the risk of invasive cervical cancer at age 66 to 80 was lower if they had a normal smear taken between ages 55 and 61, than if this was not the case (OR 0.33, CI 0.16-0.66). Women at ages 66 to 80 with a Pap smear taken within the last 5 years also had a lower risk of cervical cancer, compared to women without a Pap smear within this interval, irrespective of previous screening at ages 55 to 61 (OR 0.15, CI 0.05-0.45). Women at ages 71 to 80 showed a reduced risk of cervical cancer also if they had a Pap smear taken within the last 10 years (OR 0.21, CI 0.07-0.59).

Conclusion: A normal Pap smear between ages 55 and 61 implies a significantly lowered risk of invasive cervical cancer up to age 80. A Pap smear taken within five or ten years gives a strong protection for women at ages 66 to 80 even if no previous smear was recorded. These findings have important clinical implications. Catch up screening of older women is meaningful but should focus on women who have no smear recorded after the age of 55.

Primary HPV Testing in a Canadian Population-Based Screening Program

Objectives: To establish efficacy of high-risk HPV (HR-HPV) testing followed by liquid based cytology (LBC) triage of HPV-positives for cervical screening compared to LBC alone in an organized cervical cancer screening program; establish appropriate screening interval for HPV-negative women; determine cost-effectiveness.

Method: RCT comparing LBC to HPV testing primary screening for cervical cancer. 28,000 women 25 to 65 yrs randomized to 1 of 3 study arms until December 31, 2010. As of January 1, 2011 randomized 1:1 into Control and Intervention arms only: Control: LBC testing. Negatives screened again at 2 and 4 years. Colposcopy referral at ≥LSIL or HPV-positive ASC-US. Safety-Check: HR-HPV testing. Exit screen at 2 years with LBC. HPV-positives reflex cytology testing and managed same as intervention arm.

Intervention: HR-HPV testing. Exit screen at 4 years. HPV-positives reflex cytology testing, colposcopy referral >ASC-US. Exit colposcopy referral ≥ASC-US or HPV-positive.

Outcome measures: Cumulative ≥CIN3 detected in control and intervention arms; confirmed ≥CIN2 in control arm at 2 years; clearance of HPV infection in HPV-positives at recruitment.

Conclusions: By September 6, 2010, results available from first screening round for 16,164 subjects. Control arm: 95.2% LBC negative; 0.7% high grade squamous intraepithelial lesions (HSIL) on LBC (highest HSIL in women 25-29 yrs(2.8%)). Colposcopy referral rate 3.2%. Pathology results 12.5% CIN3. Safety and Intervention arms combined: 91.9% HPV negative. Highest HPV positivity in women 25-29 yrs (24.9%) and lowest in those 60-65 yrs (3.7%). Colposcopy referral rate 2.8%. Pathology results 14.8% CIN3.

The trial will demonstrate if HPV testing as primary screening in an organised screening program will enhance cervical cancer precursor detection, allow for extension of the screening interval, and be cost-effective.
Objective: Controversy remains over whether high-risk HPV DNA testing should be used as a primary screening test for cervical cancer. The aims of this study were to evaluate whether HPV DNA testing could be applied to cervical cancer screening programs in China, as well as other similar developing countries.

Methods: Population-based cervical cancer screening studies conducted in mainland China from 1999 to 2008, with HPV DNA testing (HC2 assay), liquid-based cytology (LBC) and visual inspection with acetic acid (VIA) concurrently performed on all women were selected for a pooled analysis. In total, 30,371 women from 17 cross-sectional, population-based studies in various parts of China were included. All women positive for any test were referred for colposcopy and biopsy. Cervical lesions were diagnosed by directed or random biopsy. The diagnostic accuracy of HPV DNA testing for detection of cervical intraepithelial neoplasia grade 3 or more severe (CIN3+) was evaluated.

Conclusions: HPV DNA testing had a higher sensitivity for detection of CIN3+ and a lower specificity compared to cytology and VIA. Sensitivity did not vary by study or age, however specificity did vary with age and was highest among women <35 years. Increasing the positive cut-point from the manufacturer recommended 1.0 to 2.0 pg/mL, decreased referral rates while slightly decreasing sensitivity. Increasing the cut-point to 10.0 pg/mL in women <35 years maintained a high sensitivity and increased specificity. HPV DNA testing is highly sensitive and moderately specific for CIN3+, with consistent results across study sites and age groups, including women <55 years. Increasing the cut-point may be beneficial for future screening programs in China, especially when screening women <55 years of age.

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The range of duration of lab utilization prior to diagnosis was 1 to 178 months (median 116 months). 9 of 56 (16%) women were diagnosed within 36 months of the onset of lab utilization. 33 of 56 had one or more Paps prior to diagnosis (median number prior to diagnosis was 3 with a range of 1 to 17). Of the 33, 18 had at least 1 negative Pap (median 3) and 11 women had 3 consecutive negative Paps prior to diagnosis at an interval of 9 to 92 months from the last negative Pap to diagnosis (median 33 months). 2 of 46,401 women with 1 or more negative cotests at age ≥ 65 were subsequently diagnosed with invasive cancer during 132,639 women-years of followup (1.5/100,000/year). Those 2 women were among the 11 who had 3 consecutive negative Paps at age ≥ 65. Most cervical cancers diagnosed at age ≥ 65 occur in women who have not met our criteria for stopping screening. A few cancers will continue to occur at age ≥ 65 despite multiple negative tests, as is true in other age groups. We currently have no evidence that these cancers would be prevented with continued screening at ages ≥ 65.
SS 10-9

10 YEARS FOLLOW UP AFTER A NEGATIVE HPV TEST.

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Introduction: HPV testing appears more and more the best tool for the cervical cancer screening. Its negative predictive value is almost perfect and it allows to increase the screening interval.

Objective: to describe the history of patients up to 10 years after HPV testing in a cytology negative population.

Material and methods: a study of 3832 women allowed in 2000 to describe the epidemiology of HPV infection in Picardy [1]. HPV DNA testing using Hybrid Capture 2 (Digene) was added to a program of liquid based cytology (Thinprep, Cytyc). Cervical pathology up to 10 years later in this cohort was searched according to the initial HPV test. In order to register cases the records of the 3 pathology departments of our territory were examined.

Results: Out of 3832 women, 3616 had cytomorphologically normal cervical smears at enrolment. Among these 3081 (85,2%) were HPV negative, 456 (12,6%) HPV positive and in 79 cases (2,5%) HPV status was unknown because of lack of residual material. Accordingly the follow up of these 3081 women is reported. We observed 58 cases of CIN2+ up to 10 years including CIN2 : 25 cases, CIN3 : 30, Micro-invasive carcinoma : 1 and invasive cancer : 1. The risk of CIN2+ is 9,21% for HPV +ve women and 0,52% for HPV –ve women. The OR is 19,43 IC : (10,83-34,87)

Discussion: These figures are lower than other of the literature [2-3] ; probably because the results don’t come of a systematic follow up but from the regional records. Nevertheless they underline the prospective interest of HPV testing

Conclusion: HPV testing well known sensitive tool for cervical screening is also of paramount prospective important interest since the risk of CIN2+ for HPV – is 0,06 at 3 years and 0,29 at 5 years.

SS 10-10

HPV SELF-SAMPLING AS AN ALTERNATIVE STRATEGY IN NON-ATTENDERS FOR CERVICAL SCREENING – A RANDOMISED CONTROLLED TRIAL

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Objectives: A randomised trial to ascertain whether women who do not attend for cervical screening are more likely to respond to the opportunity to collect a self-sample for HPV testing, or to a further invitation to attend for cervical screening. 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

Methods: The study was carried out in London in 2009. 3000 women were randomly selected from persistent non-responders (ie who had not responded to at least two invitations to attend for screening). The women were randomised on a 1:1 basis to either receive an HPV self-sampling kit or a further invitation to attend for cervical cytology. The total response in the self-sampling group for screening was 10.2%. Of the 1500 women in the control group sent a further invitation for cervical screening, 4.5% attended for cytology screening. Of the 8 women who tested positive for HPV, 7 attended for a cervical smear and had a concurrent colposcopy. Three of these (43%) had high grade disease (defined as CIN 2+), with one found to have an invasive cancer (stage 1b) and one CIN 3.

Conclusions: The value of this intervention relies on the detection of high grade CIN and early stage cancer with a good prognosis. The relatively high yield of abnormalities found is consistent with that expected among a hard to reach and relatively high risk group of women. Our study suggests that self-sampling could increase participation among non-responders in England, but further work is needed to ascertain whether the response rate seen here is likely to be representative of the rest of the country.

EUROGIN 2011 HPV associated diseases and cancer 117
SS 11-2

AGE-SPECIFIC HPV INCIDENCE AND PREVALENCE: POTENTIAL PERSPECTIVE FOR SCREENING AND VACCINATION

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Recent multi-centre epidemiologic studies of the prevalence and incidence of cervical HPV infection have been conducted via rigorous study designs that permit a cross-country comparison of the rate of infections at different ages. The often seen age-specific pattern of high prevalence soon after the onset of sexual activity at younger ages which is then followed by lower prevalence in the middle-adult years and subsequently by a second peak around menopause is not universal. Although the reason for this second, menopausal peak is not clear, it could be plausibly attributed to one or more non-mutually exclusive mechanisms, such as reactivation of the aforementioned latent infections due to a gradual loss of type-specific immunity, or to acquisition of new infections due to contacts with new sexual partners later in life. Also plausible is a cohort effect: age-related variations in prevalence may reflect the diverse HPV exposure of successive birth cohorts. Sexual mores have changed during the last few decades, which may have influenced the HPV exposure of different age cohorts. Proper understanding of the risk of cervical lesions that stem from HPV infections that are newly acquired or reactivated at different ages will help inform the implementation of HPV-based screening interventions. Studies are needed to characterize the risk of cervical precancer and cancer in populations that have a high prevalence of infections throughout the life span, such as those in Sub-Saharan Africa. If in these populations infections detected later in life are not as predictive of cervical cancer risk then HPV screening may not be as effective due to the reduced specificity at later ages.

SS 11-3

AGE RELATED HPV CERVICAL ASSOCIATED DISEASES, CONSEQUENCES FOR SCREENING, MANAGEMENT AND VACCINATION

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Probability of HPV-related cervical abnormality varies meaningfully over the age span of women. Optimally, the population-based cervical cancer screening programmes based on the cytological test should be targeted to start just few (3-5) years before the burden of invasive cervical cancer would rise in the unscreened population. This enables to gain optimal effect but also give control of potential adverse effects of the screening activities, particularly pronounced among young women. On the other hand incidence of cervical cancers as well as of mortality from the disease still continues to the rest of the lifetime of the targeted women; whereas information on in which degree the disease burden can be controlled with help of screening is still uncertain and thus more research is needed to consider how screening and management could be continued also in the old population since the last screen.

Borderline cytological abnormalities (ASC-US) are very common in women below age of 30, and their burden does not correlate clearly with the risk of developing cervical cancer. Among women below age of 35, also the rate of non-progressive CIN lesions can be very high. These have been illustrated recently using the materials from the Finnish cervical screening programme: prevalence of borderline cytology has been reported at 6%-10% and the rate of CIN treatment 0.6-1.2% per one screen in women screened at age 25, 30 or 35 years years; whereas lifetime cumulative probability of contracting cervical cancer by age 39 would have been very low, below 0.2%, in time before screening. In addition to these patterns of the natural history, one problem is that it is not yet known well whether the cytology-based screening in young women would have a similarly beneficial impact to decrease invasive cancer risk as screening e.g. at age 35 years, or more.

During the last few years, vaccination against HPV has started mainly in the well-to-do countries where the burden of the services among young women is usually very high. Vaccination will be likely to decrease the background risks of HPV infections, and of high-grade cervical pre-cancer lesions. It is not yet evident in which decree the vaccination will affect population-based rates of cancers or mild and borderline abnormalities or pre-cancers. It is likely that the impact of vaccination to borderline findings and on mild lesions is quite limited yet. Therefore, in order to avoid over-treatment and over-testing in young women, also measures to improve guidance for decisions on the screening policy are needed.
Several studies evaluated the age specific prevalence of HPV infection but more limited data are available on the age-specific incidence of new infections, especially in Europe. The NTCC trial enrolled in Italy a large population-based sample of women aged 25 to 60 years attending for regular screening after active invitation. Women in the experimental arm were tested by Hybrid Capture 2, using only the probe mix B, specific for 12 High Risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and for 1 probably carcinogenic HPV type, HPV68 classified. In five participating centres a random sample of women, HR-HPV negative by HC2 at recruitment, was re-invited for repeat HPV test at the new screening round, on average 3 years later. About 20113 women, aged 28-60, were tested for HPV at baseline in these 5 centres and a sample of 7013 of them, who were negative at baseline, was re-tested at the new screening round. We computed the age-specific proportion that was positive to Hr-HC2 and we compared it to the age specific prevalence and genotyping at recruitment in the same centres. Prevalence at re-testing was computed as weighted mean (weights inverse to sampling fractions) and compared by age at testing to prevalence at recruitment in the same centres/phases. Genotyping was performed on positive samples at recruitment and at re-testing. Among these women the proportion who tested positive at the new round decreased with increasing age from 9.9% (95%CI 6.9-12.9) among women aged 28-29 years at recruitment to 2.7% (95%CI 1.8-3.7) among women aged 40-44 years at recruitment and to 1.2% (95% CI 0.6-1.9) among women aged 55-60 years at recruitment. The ratio between the prevalence of HPV positives at recruitment and the proportion of positives at re-testing in the women who had the same age at recruitment slightly increase with increasing age although not statistically significant.

Genotyping assessment was obtained in 2,509 baseline specimens and in 344 specimens at re-testing Statistically significant differences emerged for type 16 (higher at baseline than at re-testing) and for types 51 and 68 (higher at re-testing than at baseline). These data suggest that in Italy the occurrence of new infections from high/medium risk HPV types decreases with age but that a non negligible number still occur in middle-aged women. These data also suggest lack of remarkable differences in infection persistence by age.

HPV infection is quite common with 50% acquiring HPV within 3 years after the onset of sexual activity. This vulnerability is reflected in high prevalence rates of HPV in adolescents. The vulnerability to HPV among young women is multi-factorial. Certainly, sexual risk behavior is responsible but there are also biologic reasons. HPV requires access to basal epithelial cells through a wound/ inflammation for initial infection and its replication is dependent on host cell differentiation. The transformation zone of the cervix is most active during adolescents where columnar epithelium transforms into squamous epithelium in a process referred to as squamous metaplasia (SM). SM reflects rapid cell differentiation and replication which is the perfect environment for HPV. During infection, the early proteins E6 and E7 are expressed in the parabasalar cells causing cell proliferation and as the cell matures, E4 expression causes the perinuclear halo effect. These changes reflect a LSIL or CIN 1. The natural history of HPV infection in young women show that 90% of HPV infections are cleared within 3 years. Similarly, 90% of LSIL/CIN1 in young women is also cleared underscoring the benign nature of these lesions in young women. The natural history of HSIL in young women also suggests that many of these lesions regress in young women. A recent study of CIN 2 in women aged 13-24 years found that 70% of CIN 2 regresses. Interestingly, the cervical epithelium in adolescents has different immune profiles based on topography (columnar vs. mature squamous epithelium). This constitutive immune profile appears protective and results in the high rates of clearance. Whether CIN 3 can regress in young women remains controversial. Persistent infections are reversible if appropriate adaptive immune responses develop. Factors that influence progression in adolescents include exogenous hormones which may induce cell proliferation and smoking which may dampens immune responses.

The documented high rates of regression in young women have led to new guidelines in the US which include not screening women under the age of 21 years for cervical cancer. Data suggest that the rare cancer in this age group is not prevented by the current cytologic screening methods. Second, CIN 1 and CIN 2 can be conservatively managed in young women less than 25 years of age through observation with cytology and colposcopy.
High-risk (HR) human papillomaviruses (HPVs) are now the recognized necessary cause of cervical cancer and are responsible for a significant proportion of cancers at other ano-genital sites. Infections occur most frequently in the years following sexual debut, and are very prevalent in adolescents and young adults. Because the vaccines are prophylactic and do not provide a therapeutic effect against pre-existing infections and related abnormalities, most benefits are gained by vaccinating girls before infection occurs, ideally before onset of sexual activity. Infection with an HR-HPV does not provide protection against reinfection with a different type, and may not provide protection from re-infection by the same type either. Evidence shows that older women remain at risk of HR-HPV infections throughout their lifetime. For this reason, some have argued that there may be some benefit in extending vaccination coverage to older women but the magnitude of that benefit is controversial. Most of the research in the older women group has focused on infections and not on related lesions. There is a paucity of data on the risk of lesions associated with infections in older women. Moreover, these limited data are based on prevalently-detected infections, many of which are likely to be persistent in older women, rather than incidentally-detected infections against which HPV vaccines protect. There is a variety of reasons why infections in older women may not progress to cervical precancers and cancer the same way it does in younger women, which will be reviewed: for example, the cervical epithelium has undergone metaplasia and may be less susceptible to the oncogenic effect of HPV infections; there may be some immunity from previous infections slowing the progression of disease. Until studies can better quantify the health risks associated with new infections in older women, it may be premature to recommend extending HPV vaccination coverage to this group.

**SS 11-7**

**MENOPAUSE**

**Gravitt P**

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**Objectives:** International HPV prevalence surveys have shown a second peak of HPV prevalence in women around the age of menopause in some countries (1). It is unclear whether the higher HPV prevalence in middle-aged women represents new HPV infections, re-infection, persistence, or reactivation of old infections. The HPV in Perimenopause Study (HIP) was designed to clarify the natural history of HPV in women through the menopausal transition.

**Methods:** We enrolled women age 35-60 years into a cohort study with semi-annual follow-up for 2-years from Johns Hopkins Medical Institution-affiliated gynecology clinics. We are collecting detailed information on current and past sexual behaviors of the women and their partners, hormonal medication, other medication, and tobacco/alcohol use. We are collecting detailed menstrual history to define stage of menopausal transition. Cervical secretions, swabs, and serum are collected for Pap cytology, HPV genotyping, cervical cytokine profiling, serum hormone measures and HPV serology.

**Conclusions:** We have enrolled just over 900 women with >80% retention in follow-up. The population characteristics of the cohort are favorable for our ability to better understand HPV natural history as a function of both current and past sexual behavior and the menopausal transition. Specifically, the cohort is well-screened with moderate to high socioeconomic status, less than 5% report a new sexual partner within 6 months prior to study enrollment and just over 20% report being sexually inactive in the past 6 months; the remaining report current sexual activity with one partner. More than 50% of women report having at least 5 male sexual partners in their lifetime; more than 20% report more than 10 lifetime male sex partners. Consistent with the self-reported sexual history, approximately 60% were seropositive to at least 1 of 8 HPV genotypes tested. Preliminary estimates show 18% DNA prevalence of any HPV and 9% DNA prevalence of any high risk HPV infection. Complete baseline data from this study will be presented.
P16/Ki-67 DUAL-STAINED CYTOLOGY IN PRIMARY SCREENING FOR CERVICAL CANCER AND AS TRIAGE TOOL IN PAP NEGATIVE / HPV POSITIVE CASES

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Objectives: The detection of simultaneous over-expression of the p16 biomarker protein and the expression of proliferation marker Ki-67 within the same cervical epithelial cell is an indicator of cell-cycle deregulation and thus for the presence of cervical intraepithelial neoplasia (CIN). A prospective diagnostic study (PALMS) was conducted in five European countries to assess sensitivity and specificity of p16/Ki-67 dual-stained cytology for the detection of CIN2+ (CIN grade 2 or higher) in primary screening. Furthermore, in the context of the Wolfsburg Pap cytology/HPV co-testing project the performance of p16/Ki-67 dual-stained cytology was assessed in the triage of Pap negative, HPV positive cases.

Methods and Results: 27,349 women attending routine cervical cancer screening were enrolled in the PALMS trial. Pap cytology (partially conventional, partially liquid based), HPV (hc2), and dual-stained cytology testing were performed and all women with any positive test result (except for HPV positivity <30 years as the only positive test) were referred to colposcopy/biopsy follow-up. Pathologist majority consensus diagnoses on biopsies served as gold standard. The diagnostic performance of dual-stained cytology was evaluated and compared to Pap and HPV testing. Sensitivity of p16/Ki-67 dual-stained cytology for CIN2+ was found significantly higher (90.1%) than that of Pap cytology (66.4%). Specificity was identical (95.3% vs. 95.4%). The sensitivity of HPV testing was 96.4%, but with a substantially lower specificity (90.2% over all ages) as compared to the cytology based tests. In women aged <30 years specificity of dual-stained cytology was 92.3% compared to 81.4% for HPV testing. From the Wolfsburg cohort 425 HPV positive, cytologically negative women were retested with dual-stained cytology. 25.4% of them were positive for dual-stained cytology, among them 34 of 37 histologically confirmed CIN 2+ cases were detected.

Conclusions: Dual-stained cytology testing was shown to combine both high sensitivity and high specificity for the detection of women with underlying CIN2+ in primary screening and for the triage of cytologically negative women with positive HPV test.

TRIAGE OF ASC-US AND LSIL CYTOLOGY RESULTS WITH P16/KI-67 DUAL-STAINED CYTOLOGY

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Objectives: To analyze the diagnostic performance of an immuno-cytochemical dual-staining protocol, which simultaneously detects p16 over-expression and Ki-67 expression in cervical cytology samples, for identifying high-grade cervical intraepithelial neoplasia (CIN2+) in women with Pap cytology results categorized as atypical squamous cells of undetermined significance (ASC-US), or low-grade squamous intraepithelial lesions (LSIL).

Methods and Results: p16/Ki-67 dual-stained cytology for the triage of ASC-US or LSIL was evaluated both in a retrospective (EEMAPS) and a prospective (PALMS) study. In EEMAPS, residual liquid-based cytology material from 776 retrospectively collected ASC-US or LSIL cases was used to perform p16/Ki-67 dual-staining and HPV (hc2) testing. In PALMS, dual-stained cytology was compared to HPV (hc2) testing in all women with ASC-US or LSIL (each representing 2% of all Pap cytology screening results in this prospective study). The presence of one or more double-immunoreactive cell(s) was regarded as a positive test outcome, irrespective of morphology. Test results were correlated to histology follow-up. Sensitivity of p16/Ki-67 dual-stained cytology for biopsy-confirmed CIN2+ within ASC-US was 92.2% (EEMAPS) and 94.6% (PALMS), with specificity rates of 80.6% (EEMAPS) and 77.5% (PALMS). In LSIL Pap cytology results, sensitivity of dual-stained cytology was 94.2% (EEMAPS) and 85.4% (PALMS), with specificity rates of 68.0% (EEMAPS) and 53.9% (PALMS), respectively. Dual-stained cytology showed comparable sensitivity, but significantly higher specificity when compared with HPV testing.

Conclusions: p16/Ki-67 Dual-stained cytology demonstrated a high sensitivity for the detection of underlying CIN2+ in women with ASC-US or LSIL Pap cytology results, with specificity rates significantly improved over those provided by HPV testing. In addition to the potential for improving current ASC-US triage algorithms, this novel approach may for the first time provide a triage option for LSIL cytology.
P16 IMMUNOHISTOCHEMISTRY: A POTENTIAL OBJECTIVE BIOMARKER STANDARD IN CERVICAL AND NON-CERVICAL HISTOPATHOLOGY

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Objectives: Inaccuracy in biopsy diagnosis has significant clinical implications. Despite quality training and experience there is significant interpretive variation in both diagnosis and grading of cervical biopsies. Given the mandate that whatever is biopsied should be accurately classified in order to direct the correct management, some have suggested biomarkers be routinely used to address these issues. This presentation will use our experience with p16 as a model to address issues of interpretive variability in diagnosis and grading. These data will then be generalized to potential predictive and prognostic applications in other clinical sites.

Methods: The following model is suggested for biomarker assessment: An independent masked algorithmic panel review should be used to establish a gold standard diagnosis for a large series of for example, cervical biopsies. Independently the sensitivity and specificity of immunohistochemical (IHC) assays for potential mandatory diagnostic adjuncts such as p16 and Ki-67 can be evaluated. Masking to all H&E diagnoses allows calculation of the sensitivity and specificity of each stain relative to the diagnostic gold standard. These data are compared to literature experiences pre-cancer and cancer of the cervix, other genital site tumors, squamous cancers of the head and neck and potentially other tumor types not HPV-associated.

Conclusions: In cervical biopsies, H&E diagnostic variation is significant even among experienced pathologists. Misclassification occurs in both the diagnosis of CIN vs Non-CIN as well as in grading of CIN. IHC interpretations are much less variable, but imperfect. P16 is an excellent marker of cervical pre-cancer but the complex biology of cancer may lead to some decrease in sensitivity for invasive disease. Besides aiding interpretive accuracy, p16 is establishing itself as a potential prognostic and predictive marker in cancers of the vulva, head and neck and in melanoma.
Methods: A total of 139 cervical cone biopsies were stained for the expression of p16INK4a and Ki-67 using a cocktail of two antibodies. Co-expression of Ki-67 and p16INK4a in the same cell is almost entirely restricted to HPV-transformed cervical lesions displaying diffuse p16INK4a expression, whereas in lesions with focal p16INK4a expression, p16INK4a-expressing cells are negative for epithelia transformed by HPV oncogenes. The finding will have impact also on future cytological applications of p16INK4a staining pattern, do not co-express proliferation associated Ki-67 protein, while p16INK4a positive cells in lesions with a diffuse p16INK4a staining pattern would do so.

Conclusions: Twenty of twenty-four (83.3%) metaplastic lesions displayed focal staining for p16INK4a, but all p16INK4a positive cells were negative for Ki-67. Diffuse expression of p16INK4a was observed in 12/22 (54.5%) CIN1 lesions, 11 of them simultaneously showing Ki-67 immunoreactivity in p16INK4a positive cells. Seventeen of twenty-two (77.3%) CIN2 lesions, and all 25 (100%) CIN3/CIS as well as all 46 (100%) invasive carcinoma cases displayed strong diffuse and combined expression of p16INK4a and Ki-67.

Co-expression of Ki-67 and p16INK4a in the same cell is almost entirely restricted to HPV-transformed cervical lesions displaying diffuse p16INK4a expression, whereas in lesions with focal p16INK4a expression, p16INK4a-expressing cells are negative for Ki-67. This finding confirms the association of a diffuse p16INK4a expression pattern with deregulated cell cycle in cervical epithelia transformed by HPV oncogenes. The finding will have impact also on future cytological applications of p16INK4a staining, as isolated p16INK4a positive dysplastic cells can be unequivocally identified by additional detection of Ki-67.

Results from several randomized trials argue for the implementation of hrHPV testing to increase the effectiveness of cervical screening programs. However, hrHPV positive women should be further stratified by means of triage testing to guide referral for colposcopy and minimize over-diagnosis. Currently, cytology either or not combined with HPV16/18 genotyping is considered a valuable triage tool for hrHPV positive women. However, cytology reveals a very poor sensitivity for high-grade CIN when applied to self-sampled cervico-vaginal specimens. Ideally, an objective triage method should be available that has sufficient sensitivity on self-sampled specimens as well physician-taken scrapings. This asks for novel molecular biomarkers representing essential events in cervical carcinogenesis. Various (epi)genetic profiling studies of cervical carcinogenesis have yielded such candidate biomarkers. Among these, markers detecting hypermethylation of certain genes (i.e., methylation markers) show high potential.

In previous work, we identified CADM1 and MAL as novel tumor suppressor genes functionally involved in cervical carcinogenesis. Promoter methylation showed to be the main mode of inactivation of these genes. Subsequent studies using quantitative methylation-specific PCR (qMSP) analysis on tissue specimens revealed that the application of two qMSP assays, representing CADM1 and MAL each, was sufficient to reach the highest positivity rates for CIN3 lesions (97%) and carcinomas (99%), when scoring the sum of these assays (Overmeer et al. IJC 2010). We subsequently evaluated, in independent training and validation sets of hrHPV-positive scrapes collected during population-based cervical screening studies, the potential of assessing promoter methylation of these two genes as an triage tool for hrHPV positive women. We found that, depending on the assay threshold setting, this methylation marker panel was equally discriminatory for CIN3+ as cytology or cytology with HPV16/18 genotyping, respectively, in hrHPV-positive women (Hesselink et al. Clin Cancer Res. 2011). This opens the possibility for complete cervical screening by objective, non-morphological molecular methods. Ongoing studies test the potential value of this methylation marker panel for triage testing directly on self-sampled specimens of hrHPV positive women, and monitoring of women treated for high-grade CIN.
To further improve cervical cancer screening by identifying abnormal cells associated with CIN2+ disease, a new automated test that combines the morphology of a standard BD SurePath liquid-based cervical (LBC) specimen with protein biomarker immunostaining to MCM2 and MCM7 on a single slide (hereafter referred to as SurePath Plus), was developed. This research study evaluated the performance of SurePath Plus in identifying CIN2+ disease.

The study cohort included 996 cervical cytology specimens ranging from NILM to HSIL. All LSIL and HSIL cases had biopsy results. As biopsies were not obtainable for all NILM and ASCUS cases, a negative HPV test was used as a surrogate for disease negative status. For each sample, 2 slides were produced, one prepared as a standard SurePath Pap and a second SurePath Plus slide prepared using a BD PrepStain Plus instrument that combines cell deposition with optimized immunocytochemical processing and Pap counterstaining. All slides were scored using standard Bethesda 2001 criteria. The SurePath Plus slide was further evaluated for the presence of nuclear immunostaining in morphologically abnormal cells. The distribution of cases within the various morphologic categories and their biopsy status were compared.

Comparison of the SurePath Plus slides to the SurePath Pap slides, using a cytology endpoint, resulted in a 153% increase in the HSIL+ detection rate (123 cases) and a corresponding decrease in detection rates for LSIL (23% decrease, 58 cases) and ASCUS (54% decrease, 207 cases). This increase in detection of abnormal cells correlated with the biopsy status of each case. There was a significant increase in the number of CIN2+ cases associated with HSIL+ cytology and a corresponding reduction in the amount of CIN2+ within the LSIL and ASCUS groups for the SurePath Plus Test when compared to the SurePath Pap. Specifically, the number of CIN2+ cases within the HSIL+ group increased 154% (77 cases), while the number of CIN2+ cases decreased by 62% (52 cases) within the LSIL and 62% (13 cases) for the ASCUS population.

This study reports the successful development of reagents, assay, and instrumentation that combine biomarker specific immunostaining with standard Pap counterstaining. The use of the SurePath Plus test leverages the advantages of both biomarker expression and morphologic assessment on a single LBC slide. Within this biopsy confirmed research cohort, the SurePath Plus test resulted in a more accurate detection of high grade disease.

**Objective:** The objective is to quantify the direct impact to payers in the United States of HPV OncoTect® RNA testing for ASC-US/LSIL triage in terms of clinical and economic outcomes.

**Methods:** We created a one-year budget impact model based on the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines for the management of women with abnormal cervical cancer screening tests. The model compares OncoTect testing with HPV DNA testing and focuses on women age 30 and older with ASC-US or LSIL results. We reviewed the literature for base-case estimates of model parameters, as well as estimates for use in one-way sensitivity analyses.

**Conclusions:** The base-case analysis indicates that replacing HPV DNA testing with HPV OncoTect® for women age 30 and older with ASC-US or LSIL results has the potential to save approximately $82 million annually in the United States, based on estimates of typical patient compliance following abnormal Pap or positive molecular test results. Sensitivity analyses indicate that savings could exceed $126 million with the assumption of 100% patient compliance with ASCCP guidelines. OncoTect’s high specificity would significantly reduce the number of avoidable colposcopy and biopsy procedures that follow false positive HPV DNA tests, as well as the out of pocket costs and patient anxiety associated with these procedures. In the base-case analysis, OncoTect is expected to identify 136,386 CIN2+, 56,452 CIN3+, and 2,220 invasive cancer cases annually in the population of women age 30 and older with ASC-US or LSIL results, compared with 142,585 CIN2+, 56,452 CIN3+, and 1,976 invasive cancer cases identified by HPV DNA testing. The analysis suggests OncoTect would miss 18,598 cases of CIN2+ (representing 1% of the 1,283,328 molecular tests conducted annually); the corresponding numbers for CIN3+ and invasive cancer cases are 4,249 and 0, respectively. By contrast, HPV DNA testing is expected to miss 12,399 CIN2+, 4,249 CIN3+, and 244 invasive cancer cases.

Based on the clinical performance and the projected savings associated with reduced rates of invasive testing associated with false-positive tests, use of OncoTect in high-risk patients provides the opportunity for substantial savings to payers.
Screening for Anal Intraepithelial Neoplasia (AIN) Using HPV Oncotect Increases Sensitivity and Specificity Compared HPV DNA

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Objective: High risk HPV DNA is frequently present in the anal epithelium from high risk individuals such as those with HIV co-infection yet does not always reflect whether they have an anal intraepithelial neoplastic (AIN) lesion. In this analysis of over 200 men with HIV co-infection, we hypothesized that overexpression of HPV E6, E7 mRNA would be a more specific marker of AIN than the presence of high risk HPV DNA.

Methods/Results: Two hundred and four HIV+ men were enrolled in this prospective study. Samples were collected using flocked swabs (Copan) and LiquiPrep cytology preservative. Anal cytology, HPV DNA genotyping (Linear Array®, Roche), and HPV OncoTect® (Incelldx) were compared to histology with AIN 1-3 detection used as a benchmark for performance. Sensitivity of cytology, Linear Array®, and HPV OncoTect® for AIN was 11%, 72%, and 86%, respectively. Specificity of cytology, Linear Array®, and HPV OncoTect® for AIN was 89%, 31% and 73%, respectively.

Conclusions: Overexpression of HPV E6, E7 mRNA (HPV Oncotect®) was more specific and surprisingly more sensitive than Linear Array® for AIN in HIV+ men. Anal cytology was of limited value in screening for AIN.

HPV Genome Methylation: Relationship with Cervical Disease Progression

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Persistent infection with high risk (HR) types of human papillomavirus (HPV) is strongly associated with the development of cervical cancer. Epigenetic mechanisms, such as DNA methylation, play a major role in the development of a range of cancers; however, our understanding of the role of DNA methylation in cervical cancer development is limited to a few studies of HPV types 16 and 18. Nevertheless, there appears to be a tendency towards hypermethylation of HPV16 and 18 L1 ORFs and the Long Control Region (LCR) in carcinomas and high grade lesions, but methylation is rare or absent in asymptomatic infection or low grade lesions. Differences in genome methylation status between high and low grade samples may represent a powerful biomarker for the management of HPV-associated cervical disease.

Objectives: Our objective was to determine the methylation status of CpG dinucleotides found within the LCR and L1 regions of the HPV genome using a panel (n=363) of well-characterized liquid based cytology samples with monospecific HPV16, 18, 31 or 45 infections, stratified by cervical disease grade.

Methods: A bisulfite treated DNA-based pyrosequencing and cloning approach was taken in order to estimate type-specific CpG site methylation of 3’L1-LCR-E6 for HPV16, 18, 31 and 45.

Results: Methylation estimates for HPV16 show low levels overall with a slightly higher degree of methylation in the 3’L1 region and no clear association with cervical disease stage. For HPV18, an association between hypermethylation of the 3’L1 region and increasing cervical disease stage can be seen. Preliminary results for HPV31 demonstrate low levels of methylation overall with a slightly higher degree in the 3’L1 region of high grade samples, while for HPV45 increasing levels of 3’L1 methylation appear to be associated with higher grades of disease.

Conclusions: These data suggest a possible species trend between the A9 species group (HPV16, 31), characterized by an overall low degree of 3’L1 methylation, and the A7 species group (HPV18, 45) characterized by an increasing degree of 3’L1 methylation with higher grades of cervical disease.
DETECTION AND QUANTIFICATION OF HPV E7 ONCOPROTEINS IN CERVICAL SMEARS

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The main cause for the development of cervical cancer and precancer is a persistent infection by human papillomaviruses (HPVs) of the “high-risk” group. The integration of the viral high-risk DNA into the host genome often leads to a dysregulated expression of the viral proteins E6 and E7, which are the major transforming oncoproteins of HPVs. Current cervical cancer screening relies on cytological analyses of cervical smears stained according to Papanicolau (Pap smear), which suffers from frequent false-positive and false-negative results. Our finding that E7 oncoproteins are expressed continuously in biopsies from cervical carcinomas indicates that high-risk HPV E7 proteins may be useful markers for the detection of cervical cancer and precancerous lesions. To test this prediction, we developed and characterized a set of rabbit monoclonal antibodies that detect E7 proteins from various high-risk HPVs with high sensitivity and specificity. Diagnostic tools based on these antibodies have been developed and were validated with clinical samples including cervical smears. Results with clinical samples will be presented and discussed.

THE BURDEN OF HPV RELATED HEAD AND NECK CANCER

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The incidence of oropharyngeal cancer (OPC) has increased considerably over the past decade in the Western World. This has been linked to the human papilloma virus. HPV-related OPC appears to be a distinct disease entity, which affects younger patients, and which appears to have a much better prognosis than smoking-related head and neck cancer.

In this lecture, we will discuss the evidence for the increase in oropharyngeal cancer and its association with HPV. We will discuss evidence from a recent systematic review and meta-analysis of HPV prevalence and the results of HPV incidence in a multi national, multicentre RCT giving the first worldwide study of regional prevalences. We will discuss the burden of disease worldwide and future trends.
Comparation of Molecular Methods for Detection of HPV in Oral and Oropharyngeal Squamous Carcinoma
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A variety of methods are available for the detection of HPV, each with particular strengths and limitations. The aims of the investigation using HPV measures will determine the appropriate choice of HPV assay. HPV serology can be readily adapted for use in case-control studies where a serum repository is available. HPV L1 serology will indicate cumulative exposure to type-specific HPV, though cannot differentiate past oral from anogenital infections. HPV E6/E7 serology has been used as a marker of invasive cancer. Localization of HPV to tumor cells provides the most definitive diagnostic measure, and can be obtained using p16 immunohistochemistry or in situ HPV DNA hybridization. PCR-based HPV DNA genotyping from tissue sections may be used and can cover a broad type spectrum; however methods to control for false-positive test results due to contamination of tissue are a concern. Confirmation of etiologically relevant HPV DNA first detected by PCR genotyping can be performed using HPV quantitative PCR with normalization to human DNA. HPV DNA should be present in all tumor cells, and results with low HPV to human DNA ratios are unlikely to represent HPV-positive tumors.

HPV and Clinical Heterogeneity of Head and Neck Cancer
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Head and neck squamous cell carcinomas are a major cause of morbidity and mortality, with over 500,000 cases diagnosed worldwide each year. Although previously considered one homogenous entity, data now indicate that head and neck cancers are etiologically heterogeneous. While the majority is attributable largely to tobacco and alcohol consumption, a distinct subset is attributable to oral human papillomavirus (HPV) infection. These HPV-associated head and neck cancers are distinct with regard to risk factors as well as clinical and molecular-genetic characteristics. The overwhelming risk factor for HPV-associated head and neck cancer is oral HPV16 infection that is sexually acquired. Clinically, these cancers are characterized by the presence of high-risk HPV genomic DNA in tumors (overwhelmingly HPV16), predominant oropharyngeal anatomic site, lingual and palatine tonsillar subsite, and poorly differentiated, basaloid histopathology. Additionally, HPV-positive HNSCC has an improved prognosis when compared to HPV-negative HNSCC, due in part to an improved therapeutic response to chemo-radiotherapy. HPV-positive head and neck cancers have genetic alterations that are indicative of HPV oncoprotein function and differ from HPV-negative HNSCC with regard to patterns of allelic and chromosomal loss and global gene expression profiles. In the United States and in Scandinavia, the proportion of head and neck cancers that are HPV-associated appears to be increasing over time, consistent with changes in social mores with regard to tobacco and alcohol use and sexual behavior. The newly appreciated role of HPV in head and neck cancers provides new avenues for cancer prevention and screening, provides new target for therapy, and may have important implications for the current standard of care.
HUMAN PAPILLOMAVIRUS AND HETEROGENEITY OF HEAD AND NECK CANCERS AT THE POPULATION LEVEL

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Human papillomavirus (HPV) is associated with a subset of head and neck squamous cell carcinomas (HNSCC) arising in the oropharynx, including the tonsil and base of tongue. HPV-positive HNSCCs are epidemiologically and clinically distinct compared to HPV-negative HNSCCs. Epidemiologically, HPV-positive HNSCCs are strongly related to sexual behaviors whereas HPV-negative HNSCCs are strongly related to tobacco and alcohol use. Clinically, HPV-positive HNSCCs have better overall and progression-free survival compared to HPV-negative HNSCCs. This etiologic and clinical heterogeneity of HNSCCs also manifests at the population level. In developed countries such as Australia, Canada, Japan, and the U.S., population-level incidence rates for HPV-related HNSCC sites (base of tongue, tonsil, and oropharynx) have increased substantially from the 1980s to the 2000s. In contrast, in the same countries, incidence rates for HPV-unrelated HNSCC sites (oral cavity cancers) have significantly declined during the past 20 years. Likewise, survival rates for HPV-related HNSCC sites have increased in recent years whereas survival of HPV-unrelated HNSCC sites has remained relatively unchanged. These population-level changes in morbidity and mortality for HNSCCs are believed to be caused by HPV infection and coincidental changes in smoking behaviors. The declining incidence of HPV-unrelated HNSCC sites is consistent with declines over time in cigarette smoking in developed countries. On the other hand, increasing incidence for HPV-related HNSCC sites points to increased oral HPV exposure among recent birth cohorts; presumably, from changes in oral sex behaviors. The improvements in survival for HPV-related HNSCC sites, in part, arise from an increase in the proportion of HPV-positive HNSCCs over time.

SIMILARITIES BETWEEN HPV-MEDIATED CARCINOGENESIS OF THE HEAD AND NECK AND CERVIX

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Objectives: A subgroup of head-and-neck-squamous-cell-carcinomas (HNSCC), particularly those of the oropharynx, contains high-risk human papillomavirus-type 16 (HPV16) DNA. However, only HNSCC, which in addition to PCR-detectable HPV DNA display viral oncogene (E6/E7) expression, harbour biologically relevant infections. Our aims were two-fold: A) to establish the causative effect of E6 and E7 on immortalization of primary oral epithelial cells in comparison to p53 and pRb pathway abrogation B) to determine whether HPV-DNA/ E6/E7 RNA positive SCC arising from HNSCC have specific chromosomal aberrations in common with HPV containing cervical carcinomas.

Methods: A) Primary oral keratinocytes were transduced with HPV16E6 and/or HPV16E7 either or not in combination with p53 and pRb pathway abrogation. Population-doublings and gain of immortal phenotype were determined. B) Cervical SCCs and HPV-positive and –negative HNSCC were subjected to array comparative genomic hybridization.

Conclusions: A) Expression of HPV16E6 or interference with p53 using shRNA or mutants caused an extended lifespan, whereas HPV16E7 expression or pRb pathway abrogation by knock down of p16 or Cyclin D1 overexpression had no direct effect on lifespan. Both HPV16E6 and E7 and other combinations of p53 and pRb pathway interference, caused an immortal phenotype. B) Unsupervised hierarchical clustering of genomic profiles resulted in one mainly HPV-positive cluster and one mainly HPV-negative cluster. Chromosomal gains of 3q24-29 and losses of 11q22.3-25 were common throughout, irrespective of HPV presence or organ. Within the group of HPV-positive SCC, HNSCC frequently showed gains of multiple regions at 8q, whereas cervical SCC often showed loss at 17p. Interestingly, loss at 13q21 and gain at 20q were more common in HPV-positive SCC of both organs.

Together, these data provide experimental proof for a causal association of HPV in HNSCC carcinogenesis and support a crucial role of the p53- and pRb-pathways in the transformation process. Moreover, the fact that HPV-positive HNSCC and cervical SCC share HPV-specific chromosomal aberrations points to the existence of common routes of HPV-mediated carcinogenesis at both anatomical sites.
Human papillomavirus (HPV) is a necessary but not sufficient cause of cervical cancer. A persistent infection with a high risk (hr) HPV type is associated with progression to invasive carcinomas. Due to the fact that E6 and E7 mRNA levels have been shown to correlate with the severity of the lesion, detection of E6/E7 mRNA of hr HPVs, compared with just hr HPV DNA detection, might improve the specificity of the test results for cervical precancer and cancer by reducing the false positive rate, but without reducing sensitivity for the detection of CIN 3+.

Testing methods include isothermal target amplification of E6/E7 mRNA progression markers by transcription-mediated amplification (TMA) or nucleic acid sequence based amplification (NASBA). First results demonstrate that the APTIMA HPV-Test using TMA recognizing 14 high risk HPV types has a sensitivity as high as the HC2 test and a specificity as good as thin prep cytology for CIN2+ lesions. To avoid repeated testing for the identification of the persistent active transforming infection new molecular markers are needed to predict the risk of developing cervical cancer in a onetime test. We used cervical swabs from a population based cohort consisting of 11 088 women and a median follow-up time of 12.9 years to identify possible predictive markers for persistence and progression of a HPV16 infection. Gene expression profiles of cervical swabs from (i) HPV negative women and HPV16 persistently infected and (ii) women persistently infected with HPV16 that did or did not develop progressive cervical disease within a follow up of up to 13.4 years, were compared. This led to the identification of p16/CDKN2A (p=0.039), SERPINB5 (p=0.004) and TMEM45A (p=0.0002) mRNA as potential biomarkers predicting the progression of HPV16 persistently infected women.

**Objectives:** Infections with high-risk human papillomaviruses can cause malignant transformation of the human cervical epithelium. HPV DNA tests generally are very sensitive to detect cervical neoplastic lesions but also identify transient HPV infections. As a consequence, the specificity and positive predictive value is low. Viral load assessment could improve clinical accuracy. In this study, we present the clinical accuracy of two quantitative HPV assays to identify CIN2+.

**Methods:** We analyzed viral load of all high-risk and possibly high-risk HPV types over 7 orders of magnitude (on a log10 scale) in 999 consecutive cervical smears from women participating in cervical cancer screening in Belgium enriched with ASC-US (n=100), LSIL (n=100) and HSIL (n=97) using a type-specific multiplex real-time qPCRs (1) and the BSGP5+/6+-PCR/Multiplex HPV Genotyping (MPG) assay (2).

**Conclusions:** Both assays showed a strong correlation with respect to type-specific viral load. Using an empirically determined viral load cutoff for 14 high-risk HPV types, the sensitivity for prevalent CIN2+ was reduced slightly (qPCR, from 98.4 to 93.8%; BSGP5+/6+-PCR/MPG, from 98.4 to 95.3%) compared to the minimal threshold. The specificity for absent disease (corresponding to double negative cytology at subsequent screening episodes) increased substantially (qPCR, from 89.6 to 96.2%; BSGP5+/6+-PCR/MPG, from 80.5 to 96.1%). There was no significant difference in mean viral load between LSIL and HSIL. Type-specific HPV-DNA assays show flexibility in defining thresholds and targeting HPV types, which could optimize clinical accuracy for cervical cancer precursors.
DISTRIBUTION OF THE HPV HR TYPES USING THE DIGENE HPV GENOTYPING LQ TEST IN A MULTICENTER STUDY OF A POPULATION OF ASCUS PLUS AND HC2 POSITIVE PATIENTS REFERRED FOR COLPOSCOPY: THE 3M STUDY (MILAN, MARSEILLES, AND MADRID)

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Objectives: Human papillomavirus (HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 are considered as carcinogenic to human beings. Studies have shown that a single positive test result from either type 16 or type 18 has high predictive value for CIN2+. A new Genotyping based DNA assay (digene LQ®) was recently developed and the clinical correlation with the types assessed with this assay and the severity of the disease were not fully done.
The primary aim was to assess the distribution of HPV types using this new assay in this population. The secondary aim was to correlate the HPV types to the severity of the disease with calculation of absolute and relative risk of CIN 2+ and CIN3+ according to HPV types.

Patients and Methods: The study population comprised 376 ASCUS+, HC II positive women who were admitted in three European referral gynecology clinics between 2007 and 2010: 158 patients from Madrid (Spain), 123 from Marseille (France), and 95 from Milan (Italy). The digene LQ Test utilizes probes for 18 HR HPV types (i.e., HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73, and 82). The digene LQ test was performed in the Luminex 100 IS System (Luminex Corporation). The samples from three different sites will be analyzed in a centralized laboratory (Alphabio, Marseille).

Results: The respective distributions of the HPV types using the digene LQ® were: HPV 16 (40%), HPV 18 (7%), HPV 31 (17%), HPV 33 (6%), HPV 35 (2%), HPV 39 (3%), HPV 45 (3%), HPV 51 (6%), HPV 52 (5%), HPV 53 (3%), HPV 56 (7%), HPV 58 (6%), HPV 59 (1%), HPV 66 (5%), HPV 68 (3%), HPV 73 (1%), HPV 82 (1%), and multiples HPV infection in 18% of cases and 7% of negative results.
The absolute risk of being CIN2+ when having HPV16 is 58% similar to the risk associated with HPV 31 – 52% and the risk is 28% if HPV18 positive. The risk of being CIN3+ when having HPV16 is 23% (the risk is 3% if HPV18 positive, and 13% if HPV31 positive). The relative risk of CIN2+ when being HPV16+/HPV18- is 58% (the risk of CIN3+ is 24%). Similarly, the risk of CIN2+ when being HPV16+/HPV31- is 59% (the risk of CIN3+ is 25%), and the risk of CIN2+ when being HPV31+/HPV18- is 54% (the risk of CIN3+ is 13%).

Conclusions: The digene LQ®, a new sequence-specific Hybrid Capture sample preparation is fast, efficient and allows high-throughput genotyping of 18 HR HPV types by PCR compared to traditional non-sequence-specific sample preparation methods. This study showed that HPV type 16, 31, 33, 18, and mixed genotypes is the most prevalent genotypes and that ASCUS plus women HPV 16 positive have the highest absolute and relative risk of CIN2+ and CIN3+.

HPV DNA TESTING AND CYTOLOGY AS PREDICTORS OF HISTOLOGICALLY CONFIRMED CERVICAL DISEASE: EVIDENCE FROM THE LUDWIG-MCGILL COHORT

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Objectives: Although many studies have compared human papillomavirus (HPV) DNA testing to cervical cytology for the detection of pre-invasive lesions of the cervix, most used cytology results as an endpoint, thus being subject to outcome misclassification. Our objective was to use the gold standard of histopathology to ascertain lesion outcomes in a repeated-measurement, longitudinal study of HPV and cytology testing.

Methods: A cohort study was conducted in Brazil and enrolled 2462 women for interviews, cervical cytology, cervicography and HPV testing according to a pre-established protocol. HPV DNA testing and cytology were performed at each visit, i.e., every 4 months in the first year and every 6 months in the subsequent years. Whenever high-grade lesions were detected on Pap cytology or on cervicography, subjects were referred for colposcopy and underwent biopsy if applicable. The specificity, sensitivity and predictive values of HPV DNA testing and cytology, both cross-sectionally and as repeated longitudinal measurements, were calculated and summarized in receiver operating characteristic (ROC) analyses. In addition, time-to-event analyses using Kaplan-Meier plots and Cox regression were performed comparing screening modalities.

Conclusions: Among the 355 women referred for colposcopy, 86 were diagnosed with cervical intraepithelial neoplasia (CIN) on histology during follow-up. The incidence rate of CIN per 1000 women-months increased from 1.13 (95% confidence interval (CI): 0.76-1.68) in women never exposed to HPV, to 2.46 (95% CI: 1.02-5.91) in women exposed only to low-risk HPV types, to 5.65 (95% CI: 4.25-7.49) in women exposed to at least one high-risk HPV type. When considering cytology screening, incidence rates increased from 1.34 (95% CI: 1.01-1.79) in women with negative results, to 5.46 (95% CI: 2.05-14.54) in women with atypical squamous cells of undetermined significance (ASCUS), to 9.05 (95% CI: 5.25-15.59) in women with low-grade squamous intra-epithelial lesions (SIL) and finally to 15.31 (95% CI: 10.17-23.04) in women with high-grade SIL. Our results emphasize the importance of combining HPV DNA testing with cytology for an optimal screening model.
A COMPARISON OF HPV TYPE-SPECIFIC RESULTS AMONG WOMEN WITH ABNORMAL SMEARS

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Objectives: This study aims to investigate the prevalence of HPV types in women referred with an abnormal smear and the positive predictive value (PPV) of several HPV typing tests for the detection of CIN2+ and CIN3+.

Methods: A total of 779 women in PREDICTORS 1 and 934 women in PREDICTORS 2 were included. Typing was performed in PREDICTORS 1 using Abbott (HPV 16, 18), Norchip Pre-Tect Proofer (HPV 16, 18, 31, 33, 45), Linear Array (37 types) and in-house qPCR (HPV 16, 18; plus 31, 33, 35, 51, 52, 58 if initially 16 and 18 negative). Typing was performed in PREDICTORS 2 using Abbott, Norchip Pre-Tect Proofer, APTIMA (HPV 16, (18,45)), BD (HPV 16, 18, 31, 45, 51, 52, 59, (33,56,58,66), (35,39,68)) and Cobas (HPV 16, 18). We present PPV and relative detection rate (type positive detected by test / type positive on any test).

Conclusions: Prevalence of CIN2+ and CIN3+ in PREDICTORS 1 was 29.4% and 21.3% respectively. For PREDICTORS 2 these were higher at 32.4% and 23.8%. HPV 16 was detected by at least one test for 33.2% women in PREDICTORS 1 and 33.9% in PREDICTORS 2; HPV 18 without 16 for 9.9% in PREDICTORS 1 and 10.8% in PREDICTORS 2; HPV other without 16 or 18 for 50.3% in PREDICTORS 1 and 48.1% in PREDICTORS 2. For PREDICTORS 1, PPV (relative detection rate) for CIN2+ for HPV 16 was 54.8% (93.1%) for Linear Array, 56.4% (86.9%) for Abbott, 57.0% (88.8%) for qPCR and 59.5% (75.3%) for Norchip; for PREDICTORS 2, 56.7% (89.6%) for APTIMA, 56.8% (95.6%) for Cobas, 56.9% (91.5%) for Abbott, 58.1% (91.2%) for BD and 64.5% (77.3%) for Norchip. PPV (relative detection rate) of HPV type 18 (without type 16) for PREDICTORS 1 was 26.3% (74.0%) for Linear Array, 28.8% (67.5%) for Abbott, 33.3% (81.8%) for qPCR and 34.3% (45.5%) for Norchip; for PREDICTORS 2, 30.2% (62.4%) for BD, 31.3% (63.4%) for Cobas, 31.6% (78.2%) for APTIMA, 33.9% (55.4%) for Norchip and 35.8% (52.5%) for Abbott. PPV (relative detection rate) of any other HPV type (without type 16 or 18) for PREDICTORS 1 was 26.3% (74.0%) for Linear Array, 28.8% (67.5%) for Abbott, 33.3% (81.8%) for qPCR and 34.3% (45.5%) for Norchip; for PREDICTORS 2, 30.2% (62.4%) for BD, 31.3% (63.4%) for Cobas, 31.6% (78.2%) for APTIMA, 33.9% (55.4%) for Norchip and 35.8% (52.5%) for Abbott.

COMBINED MORPHOLOGIC AND MOLECULAR ASSESSMENT OF CERVICAL CELLS IN SUSPENSION-THE SLIDELESS PAP SCREEN

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Background: Primary screening for cervical cancer using HPV DNA has been controversial due to the low specificity of HPV DNA for high grade lesion by biopsy. Another impediment to widespread adoption of HPV as a primary screening test has been the body of information and increased specificity provided by morphologic assessment of cervical cells on a slide (PAP).

Methods/Results: Using the HPV OncoTect® E6, E7 mRNA quantification kit which includes the nuclear stain DAPI, we performed “The Slideless PAP” on 100 liquid based cervical cytology specimens using a hybrid instrument that provides morphologic assessment including nuclear pleiomorphism/condensation, nuclear/cytoplasmic ratio as well E6, E7 mRNA overexpression and DNA ploidy analysis. This assessment correctly categorized 60 normal cytology samples, 29 LSIL samples, and 10 HSIL samples (99% accuracy). One LSIL samples was categorized by our system as high grade based on a nuclear/cytoplasmic ratio >0.5 and E6, E7 mRNA overexpression with nuclear aneuploidy. Women are currently being followed for biopsy correlation.

Conclusion: This paradigm changing system combines and digitizes the criteria used by pathologists to determine cytologic abnormalities in liquid-based cytology specimens with E6, E7 mRNA quantification/cell and DNA ploidy analysis. This system is highly automated with a 96-well format and walk away sample analysis. Images are created in 3-D (without slides) in less than 1 minute per sample and no nucleic acid extraction is necessary.
HPV is the most common sexually transmitted infection (STI), and most sexually-active persons acquire it over a lifetime. Despite the fact that HPV is transmitted between sexual partners, the vast majority of epidemiologic research has been individual-based, and typically focused on women. Far less research has been conducted among men or couples. To understand the spread of HPV in populations, transmission studies are needed. These are ideally conducted in couple-based studies. Several of the latter investigations have begun in different populations and have advanced our understanding of behavioural and viral characteristics that influence the probability of heterosexual transmission. Knowledge of transmission parameters per act and per partnership will provide empirically valid modeling of the cost effectiveness of HPV-based interventions, such as screening and vaccination. With the discovery that microbicides such as carrageenan can be inhibitory against HPV in vitro, trials in couples will assist in obtaining clinical evidence of the putative efficacy of vaginal gels in preventing HPV infection.

We have developed a mouse cervicovaginal challenge model for HPVs based on our previous development of a procedure for efficiently generating HPV capsid-based gene transfer vectors, call pseudovirions (PsV). We found that the PsV cannot infect, or even bind, intact stratified squamous epithelium of the vagina and ectocervix or intact simple columnar epithelium of the endocervix. In order to establish epithelial infection, the viral L1 major capsid protein must first bind to heparan sulfate proteoglycans (HSPG) on the basement membrane (BM) that separate the dermis and epidermis. The BM becomes exposed at sites of epithelial trauma or permeabilization. As we previously determined for cultured cells, the bound capsid must then undergo a conformational change that permits furin cleavage of the N-terminus of L2, the minor capsid protein. L2 cleavage leads to exposure of the L2 cross-neutralizing epitopes. We believe that this conformational change in L2 exposes a site on L1 that can now engage an as yet unidentified keratinocyte specific receptor, leading to infection of keratinocytes as the migrates over the BM to close the “wound”. Only keratinocytes become infected in this model of cervicovaginal infection. In contrast to primary keratinocytes in vivo or in vitro, established “normal” and tumor-derived epithelial lines have cell surface HSPGs that allow the conformational change needed for transfer to a second receptor and internalization. An understanding of this difference explains why L2 neutralizing antibodies are more effective in vivo than in vitro and has provided us with the critical insights for developing a more sensitive in vitro assay for L2 neutralizing antibodies.
Viral latency is usually referred to states of where the virus is able to lie dormant within a cell. A latent viral infection is a type of persistent viral infection which is distinguished from a chronic viral infection. In the case of latency, the virus production ceases but the viral genome continues to exist. The latent virus can be reactivated and begin to produce large amount of virus without the host being infected by new virus. Examples of latent virus is HSV. Virus latency should not be confused with clinical latency such as influenza which there are no clinical symptoms during the incubation period. Whether HPV has true latency remains controversial. Certainly, HPV appears to clear among most women defined as repeated negative tests HPV DNA. On the other hand, studies have shown that there can be intermittent detection of HPV DNA in some. The intermittent negative tests may reflect re-infection from a partner who carries HPV, viral latency, viral replication below the level of detection of a test, or simply bad sampling. We know that cytology can be quite insensitive because it relies on a relative random sampling technique. Sampling for HPV DNA may have similar limitations. Some countries have shown that there is an upsurge of prevalence in women around their 50’s. Whether this is viral reactivation or introduction of new infections is unknown. Most of these studies are limited since “partners” are not tested and their fidelity remains questionable. The high rates of HPV in HIV infected women have led many to believe these are reactivations of previous infections. However, most of these women have not been abstinent making these conclusions difficult. Certainly, HIV infected women have difficulty in clearing HPV, however, most HIV infected women were sexually active at the time they acquired HIV. In 1,500 young women, we examined patterns of re-detection of HPV. In this cohort we found that around 11% had recurrence of the same type including 16, 52, 54, 59, 66, 68, 31, 45, 18, 58, 51, and 66. In women who had an incident/prevalent HPV 16 infection and had greater than 5 years of follow-up; approximately one quarter showed recurrence. Of these women, another quarter had persistence. The other potential source of these “reinfections” may be the anus or vagina. Our data suggest that the majority of these may be re-exposure (from partner, anus or vagina) with rapid clearance due to a cell mediated immune response. However, a small portion of these are likely the same ongoing infection. Intermittent negative tests may be due to low levels of viral DNA, but likely it is not a true latency.

MOLECULAR PHENOTYPES OF HPV GENOTYPES
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We have analyzed E6 proteins of 19 human papillomaviruses (HPV) able to infect genital tissue with regard to their ability to degrade p53 and the thus far unknown immortalization potential of the genomes of HPV 53, 56, 58, 61, 66 and 82 in primary human keratinocytes. High-risk types E6 proteins of HPV types 16, 18, 33, 35, 39, 45, 51, 52, 56, 58 and 66 were able to induce p53 degradation in vitro, and HPV18-, HPV56-, and HPV58-immortalized keratinocytes revealed markedly reduced levels of p53. In contrast, the E6 proteins of HPV6 and 11 and HPV44, 54, and 61, regarded as low-risk HPV types did not degrade p53. Interestingly, the E6 proteins of HPV 53, 70 and 82 inconsistently risk classified in the literature were also found to induce p53 degradation. The genomes of HPV53 and 82 immortalized primary human keratinocytes that revealed almost absent nuclear levels of p53. These data suggest a strict correlation between the biological properties of certain HPV types with conserved nucleotide sequence (phylogeny), which is largely coherent with epidemiologic risk classification. HPV types 16, 18, 33, 35, 39, 45, 51, 52, 56, 58 and 66, generally accepted as high-risk types, behaved in our assays biologically different from HPV types 6, 11, 44, 54 and 61. In contrast, HPV70, regarded as low-risk type, and HPV53 or HPV82, with inconsistent described risk status, were indistinguishable with respect to p53 degradation and immortalization from prototype high-risk HPV types (1). This could imply that other important functional differences exist between phylogenetically highly related viruses displaying similar biological properties in tissue culture that may affect their carcinogenicity in vivo. We therefore investigated other E6 targets that bind via a PDZ domain to the C-terminus of E6. Our results show that E6 proteins from low-risk HPV70 and possibly high-risk HPV82 interact and degrade PDZ proteins hDlg and Magi1 identical to HPV16E6 and HPV18E6. In contrast high-risk HPV66E6 did not bind or degrade hDlg or Magi1 (2). Together with our data related to p53 degradation, this shows that neither binding of E6 to p53, to E6AP, to Magi1 and hDlg, the degradation of hDlg and Magi1, nor immortalization of normal human keratinocytes seems to be a reliable predictor for carcinogenic behaviour of HPV in the cervix.

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HPV is thought to be a highly transmissible infection, however, to date, few prospective studies have been conducted and none have provided estimates of the per contact or per relationship rate of transmission. The majority of published studies are cross-sectional and among heterosexual partners. Overall the rate of HPV concordance among heterosexual partners is from 22.7% to 76% when examining concordance for any type of HPV. Type specific HPV concordance appears to be lower with a reported range of 27% to 58%.

The range in HPV concordance is likely influenced by the disease status of the male and females in the partnership as well as the timing of the assessment of sexual behavior relative to the timing of the HPV measurement. In a study that examined very recent sexual behavior in relation to HPV type concordance among 25 couples, a concordance of 68% was observed (Widdice 2010).

Results from the few published prospective studies indicate that multiple HPV transmission events occur within a sexual couple, HPV appears to be more efficiently transmitted from the female to the male compared to the male to the female (Hernandez et al 2008), and adult male circumcision significantly reduces HPV transmission to the female partner (Wawer et al 2011).

Utilizing cross-sectional data, Burchell and colleagues approximated the probability of heterosexual HPV transmission. Using this approach they estimated the transmission probability to be between 0.05-1.0 with a median probability of 0.4. These estimates are significantly higher than observed from actual transmission studies of other STIs such as HIV and HSV where probability of transmission per coital act was 0.00089 for HSV. Altogether these reports indicate that HPV type concordance is highly variable (22.7% - 76%) with a highly variable rate of transmission. This variability may be due to differences in rates of HPV acquisition between men and women as well as differences in duration of infection.

Objectives: Genital-to-genital and penis-to-anus HPV transmission is well-established. However, evidence is emerging for alternative routes of genital HPV transmission.

Methods: The acquisition of an anal human HPV infection is a relatively common event among women. Seventy percent of women participating in the Hawaii HPV Cohort study tested positive for anal HPV infection at 1 or more clinic visits during an average 1.3 year follow-up period [1]. A high degree of genotypic concordance in the sequential acquisition of cervical and anal HPV infections was observed: women with the same genotype observed previously in the adjacent anatomic site (ie, cervix or anus) were significantly more likely to have an incident anal or cervical HPV infection than were women with a discordant HPV type or no previous HPV infection [2,3]. The risk of acquisition of a concordant anal HPV infection following a cervical infection with the same HPV type was especially high, suggesting that the cervix (vagina) may serve as a reservoir for anal HPV infection. In a pilot study, the anus of women was both a major source and target of heterosexual transmission [4]. We observed consistency between penis-to-female anus transmission and reported anal intercourse during the corresponding period. Transmission through non-penetrative sexual contact was demonstrated between the female anus and the scrotum, as well as between the female hand and male genitals. Male self-transmission frequently involved the scrotum, likely facilitated by passive contact between proximate genital sites. The scrotum may be an important reservoir of infection for penile infections that can subsequently be transmitted to partners. Hands may also serve as reservoirs of infection in both men and women. Autoinoculation involving the hands may result from casual contact or masturbation.

Conclusions: The development of comprehensive HPV prevention and control strategies, which incorporate HPV vaccine usage and contraceptive practices, is impeded by lack of information on the risk and routes of sexual transmission between heterosexual and homosexual partners, and potential genotype-specific differences in transmission efficiency.
SS 16-7
RECENT ADVANCES AND GAPS IN RESEARCH: CONCLUSIONS
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It is now well established and widely, if not universally, accepted that virtually all cervical cancer and its immediate precancerous lesions arise from persisting cervical infections by approximately 15 cancer-associated (carcinogenic) human papillomavirus (HPV) genotypes. Based on this nearly absolute etiologic link between carcinogenic HPV and cervical cancer, two new approaches for the prevention of cervical cancer have emerged:

1) HPV vaccination for primary HPV prevention in younger women and
2) carcinogenic HPV detection for secondary prevention via identifying and treating cervical precancer and early cancers.

Both have demonstrated high degrees of efficacy with maximum effectiveness guided by an understanding of the causal model and application of these technologies in an age-appropriate manner. Despite these novel cervical cancer prevention tools, there are still important gaps in research that if bridged could be translated into new tools to reduce the burden of cervical cancer, the third most common female cause worldwide:

1) Lack of detailed knowledge of what constitutes an effective natural immune response for the clearance of HPV infections, which would inform the development of HPV immunotherapeutics;
2) Unknown genetic basis for the variability in carcinogenicity of HPV genotypes, which would provide a rational approach to anti-viral therapies; and
3) Poor understanding of the molecular events leading to HPV persistence and progression to precancer, which would provide the leads to identifying biomarkers that differentiate between benign and clinically important HPV. Finally, the development of new intervention tools must adapted to low-resource settings, where more than 80% of cervical cancer occurs.

SS 17-1
THE DURATION OF INFECTION AND CLEARANCE OF HPV 6, 11, 16 AND 18 IN MEN AGED 16 TO 26
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Background: Despite the large body of knowledge of HPV natural history accumulated in women, little is known about the natural history and the rate of acquisition and clearance of HPV infection in men. In this analysis we examine the incidence and clearance of external genital HPV infection in men aged 16 to 26.

Methods: A total of 2033 men between ages 15 and 27 in the placebo arm of a quadrivalent HPV vaccine clinical trial were included in this analysis, including 1732 heterosexual men (HM) and 301 men who have sex with men (MSM). These men were recruited from 14 countries in Africa, Asia, North and South America, and Europe. Subjects underwent detailed anogenital exams as well as sampling from the penis, scrotum and perineal/perianal area at enrollment and at regular intervals afterwards. Subjects who were PCR negative to either HPV 6, 11, 16 or 18 at enrollment were considered in the incidence analysis of that HPV type. Incidence was analyzed either as DNA detection (DNA detected via PCR at one visit) or persistent infection (DNA detected via PCR at two visits at least 6 months apart). Subjects who were PCR positive to HPV 6, 11, 16 or 18 at enrollment were considered in the clearance analysis for that HPV type. Clearance was defined as negativity to a specific HPV type on two consecutive visits. If a subject was diagnosed with disease related to HPV and then cleared their infection based on the definition above, he was not counted as a case of clearance.

Results: The rate of HPV 6, 11, 16 or 18 DNA detection and persistent infection was 11.1 and 5.2 per 100 person years. The incidence of both DNA detection and persistent infection was higher in MSM subjects (as opposed to HM subjects) (20.9 vs 9.6 for DNA detection and 11.4 vs 4.2 for persistent infection, respectively). In subjects who were PCR positive and seronegative for the respective HPV type at enrollment, the rate of clearance of HPV 6, 11, 16, and 18 was 39.3, 77.2, 42.8 and 57.2 per 100 person years, respectively. Duration of infection data were not available at the time of writing, but will be presented as well.

Conclusions: The study results suggest that the acquisition of HPV 6, 11, 16, or 18 in men is common, particularly among MSM. Additionally, many of these HPV infections are also subsequently cleared, similar to data available in women. Nevertheless, given the high rate of new HPV infections in young men, male HPV vaccination may reduce infection in men and help to increase community level herd immunity.
INCIDENCE AND COSTS OF ANAL, PENILE, VAGINAL AND VULVA CANCER IN DENMARK

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Objectives: Apart from genital warts and cervical cancer human papillomavirus (HPV) also is attributable for 40%-91% of all cases of anal, penile, vaginal and vulva cancer and precancerous lesions and among these cases 60%-90% is attributable to HPV 16 & 18. Overall the number of new cases of these four cancers may be relatively high implying notable health care cost to society. It is the purpose of this study to estimate the incidence and the health care sector costs of anal, penile, vaginal and vulva cancer.

Methods: New cancer patients were identified via specific ICD-10 diagnosis codes in the Danish National Cancer Register. Their resource use in the health care sector 1, 2, and 3 years after the time of diagnosis were estimated on the basis of data from the National Patient Register via the individual patient’s unique registration number (CPR-No.). Resource use in the hospital sector is defined in terms of registered hospital contacts for which there is a description of the resource use associated defined according to the DRG-system. DRG-charges were used as cost estimates. For the cohort of cancer patients diagnosed during 2004-2007 healthcare consumption in 2008 was compared with a cohort free of cancer. Healthcare costs attributable to the four cancers were estimated by regression analysis.

Conclusions: The incidence rate of penile cancer in Denmark is 1.7 per 100,000 persons (equal to 50 new cases). The corresponding incidence rates for anal, vaginal and vulva cancer are 1.9, 0.9 and 3.6 per 100,000 persons, respectively. In total, the number of new cases of these four cancers in Denmark is 285 per year. In comparison, the total number of new cases cervical cancer is almost 400 per year. Preliminary results show for example that the cost of penile cancer per patient is €12,242 the 1st year, €3,980 the 2nd year, and €3,435 the 3rd year after the time of diagnosis, corresponding to annual hospital costs of penile cancer at €920,000 in Denmark. It is expected that the Danish HPV vaccination program currently in place will significantly reduce this burden. This study provides the first estimate of the burden associated to non-cervical HPV-related cancers in Denmark based on very reliable individual-based data.

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INCREASED RISK OF MOTHER TO CHILD TRANSMISSION OF HPV IN HPV POSITIVE MOTHERS

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Background: Currently, HPV research focuses on HPV infection in adults, with sexual transmission as the main route of infection. However, data on HPV infection in children is slowly becoming available (for review: Syrjanen et al., 2010). Furthermore, it is currently a matter of debate if mother-to-child transmission of HPV has to be considered an important infection route and whether children born out of HPV positive mothers are at higher risk to be HPV infected. The objective of this meta-analysis is to clarify and summarize published literature on the extent to which cervical HPV infection can be transmitted from mother to child.

Methods: Medline, Web of Science, CINAHL and ELIN were systematically searched for eligible publications until December 2010. Articles were selected based on strict inclusion and exclusion criteria. After testing for heterogeneity of studies, meta-analysis was performed using the random effect model.

Results: Twenty one eligible studies were selected to review the risk of newborns from HPV positive mothers to be infected with HPV as well, in total including 3128 women/children pairs. High heterogeneity could be found, I²=96,37%. The overall estimated risk difference was 0.33; 95% confidence interval, 0.22-0.44 (p<0.001). Restricted to studies including high risk HPV positive mothers only (n=5; #women=539), the risk difference was 0.35; 95% confidence interval, 0.04-0.66 (p<0.001). Heterogeneity was found to be high I²= 88,93%, mainly due to the effect of one study. Excluding this study (Puranen et al., 1997) strongly reduced the heterogeneity (I²=14,89) and the risk difference went up to 0.45; 95% confidence interval, 0.33-0.56 (p<0.001).

Discussion: This meta-analysis of published literature indicates an elevated risk for children born out of HPV positive mothers to be HPV positive themselves. Potential explanations include a higher contamination rate during early nursing from mother to child or vertical transmission of HPV during pregnancy and/or birth giving. More research is required to gain insight mode of infection of mother to child.
**Background**

Human Papillomavirus (HPV) genotype distribution in invasive cervical cancer (ICC) is critical to guide the introduction of HPV prophylactic vaccines.

Histological confirmed ICC cases from 38 countries were assembled. HPV detection was done by polymerase chain reaction using SPF-10 broad-spectrum primers followed by deoxyribonucleic acid enzyme immunoassay and genotyping by reverse hybridization line probe assay (LiPA25) (version 1). Of 10,575 ICC cases, 8,977 were HPV-DNA positive (84.9%). The most common types were HPV 16, 18, 45, 33, 31, 52, 58 and 35, with a combined worldwide relative contribution of 91.3% (95% confidence interval (CI)=90.7%-92.0%). HPV 16, 18 and 45 were the most relevant type among young women and among adenocarcinomas. These data suggest that type specific screening should consider the inclusion of HPV 45 in addition to HPV 16 & 18.

**Objectives.** Co-infection with Chlamydia trachomatis and human papillomavirus (HPV) is likely to be common. Infection with either one may increase the risk of a co-infection, while clearance of either infection could be affected by a concurrent infection as well. We used data from women participating in two consecutive rounds of a Chlamydia screening program to determine the percentage of (type-specific) HPV infections among women co-infected with Chlamydia or not, and to assess the association of Chlamydia on the prevalence of type-specific HPV infections.

**Methods.** Between 2008 and 2010, 45,000 females aged 16 – 29 years participated in a large scale Chlamydia screening trial, by self-collecting a vaginal swab for Chlamydia PCR-testing. We selected 3470 females who had given informed consent to use their sample for further (anonymous) tests and also had filled in a voluntary questionnaire about their sexual behaviour. Of these 72% (n=2496) participated in both rounds, with about 1 year between both rounds.

HPV detection was performed retrospectively by PCR using SPF10 primers and LiPA genotyping (DDL, the Netherlands). HPV genotype specific prevalences were determined; evaluation of the effect of Chlamydia on HPV prevalence and persistence corrected for risk factors and age is ongoing.

**Results.** The Chlamydia prevalence was 4% (n=150) among the selected 3470 females. Preliminary results of 2682 females tested for the presence of HPV, showed a HPV prevalence among Chlamydia positive and negative females of 77% (n=41/ N=53) and 61% (n=1602/ N=2629), respectively (p=0.02). Of the initially Chlamydia positive females participating in both rounds 74% (n=14/ N=19) was HPV infected in the 1st round and of these 79% (n=11/ N=14) was still HPV positive in the 2nd round. For Chlamydia negative females this was 60% (n=543/ N=911) and 88% (n=476/ N=543), respectively. Comparison of HPV genotypes between both rounds will enable distinguishing between re-infection and persistence and exploring differences between HPV-types in Chlamydia positive (n=95) and negative (n=2401) females.

**Conclusions.** HPV prevalence was higher in Chlamydia positive females than in Chlamydia negative females. Based on descriptive analyses of preliminary data no effect of a Chlamydia infection on the prevalence of HPV a year later is expected. More in depth analyses on the total selected population of 3470 females will be presented at the conference.
Objective: China is currently classified as having low cervical cancer risk. This designation is based on data from published cancer registries, which may not be representative. Human papillomavirus (HPV) prevalence has been shown to correlate well with cervical cancer prevalence in any given population. Using individual patient data from 17 population-based studies in 9 Chinese provinces, we aim to establish more accurate rates of HPV and CIN prevalence in China.

Methods: 29,488 women received HPV DNA testing (HC2, Qiagen Inc), visual inspection with acetic acid, and liquid-based cytology. Women positive for any test received direct biopsy for visible lesions or 4-quadrant biopsy. The diagnostic gold standard was histological-confirmed biopsy.

Conclusions: 28,889 women had HPV testing results. The prevalence of high-risk HPV (HR-HPV) was 15.2% (95% CI:13.0-17.7%) and the age-standardized HR-HPV rate was 15.6% (95% CI:15.2-15.8%). Age-stratified pooled HR-HPV prevalence peaked at ages 15-24 years (17.2%, [95% CI:14.6-20.2%]) and 40-44 (16.6%, [95% CI: 13.9-19.6%]). For women with normal cytology, the overall age-standardized HR-HPV prevalence was 9.2% (95% CI: 8.9-9.4%). 28,063 women had cytology results. The prevalence of CIN2 was 1.3% (95% CI:1.0-1.6%) and CIN3+ was 1.3% (95% CI:0.9%-1.7%). Age-standardized rates were 1.8% (95%CI: 1.7-1.9%) for CIN2, and 1.6% (95%CI:1.5-1.7%) for CIN3+. Age-stratified CIN2 prevalence was constant with age, while CIN3+ prevalence peaked at ages 15-24 (1.5%, 95%CI: 0.7-2.9%) and 40-44 (2.0% [95%CI: 1.4-2.8%]). This is the largest study of HPV and CIN prevalence in China conducted independently of published Chinese cancer-registries. Our age-standardized HR-HPV prevalence in China is higher than previously reported, and our HR-HPV prevalence remained high in women ≥40 years. These findings suggest the cervical cancer risk in Chinese women may be underreported.

**Risk Factors for HPV Infection in Women in Portugal – The Cleopatre Portugal Study**

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Objectives: One of the secondary objectives of the CLEOPATRE Portugal study was to investigate the potential for demographic, socio-economic, lifestyle and medical history as possible risk factors to predict cervical HPV infection in women in mainland Portugal.

Methods: This cross-sectional study recruited women aged 18–64 years, according to an age-stratified sampling strategy, at selected gynaecology/obstetrics or STD clinics across the five regional health administrations (ARS) of mainland Portugal. Cervical liquid-based cytology samples were sent to central laboratories for HPV genotyping (Clinical Array HPV 2 assay) and cytological diagnosis. Univariate and multivariate logistic regression analyses were conducted to identify risk factors for HPV infection. The potential risk factors evaluated were age, ARS, country of birth, level of education, smoking habits, age at first sexual intercourse, contraceptive use, lifetime number of sexual partners, current partner’s circumcision status, history of cervical intraepithelial lesions, any STD in the previous 12 months, and current status of the immune system. Descriptive and inferential analyses were performed using SPSS software.

Conclusions: Among the 2326 women included (crude HPV infection prevalence was 19.4%), lifetime number of sexual partners was a strong predictor of HPV infection, with a 5.4-fold higher risk of infection among women reporting 5–10 partners compared with those reporting only one partner. Other risk factors were younger age (highest risk in 20–24 years-old), country of birth other than mainland Portugal, education to secondary school level, smoking history ≤10 years, and an STD within the previous 12 months. The most important risk factors for multiple versus single infection were age 60–64 or 20–24 years, and region covered by ARS Centre or Lisbon. The only identified risk factor for infection with at least one high-risk type versus low-risk types was contraception with condoms or oral contraceptives. These data from the first population-based HPV prevalence study in Portugal will contribute to a better understanding of the wide spectrum of HPV infection across Europe and may help to identify women at increased risk of HPV infection.
THE ROLE OF COFACTORS IN PROGRESSION OF CERVICAL PRECANCEROUS LESIONS

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Objectives: Several environmental cofactors have been recognized as potential risk factors for precancerous lesions and cervical cancer; however, there is little understanding of when these factors play a role in the natural history of disease. Tobacco smoking, oral contraceptive use, and parity have been identified previously as important risk factors for cervical cancer. We studied the role of these factors in progression of disease from a lower to a higher grade of cervical intraepithelial neoplasia (CIN) and further to squamous cell carcinoma (SCC).

Methods: From the ongoing Biomarkers of Cervical Cancer Risk case-control study of Montreal women, we obtained self-reported information about environmental cofactors for human papillomavirus (HPV) positive women with normal cytology (n=279) and women with histologically diagnosed CIN 1 (n=60), CIN 2 (n=177), CIN 3 (n=268) and SCC (n=137). Odds ratio (OR) estimates and 95% confidence intervals (CI) for the association between tobacco smoking, oral contraceptive use, and parity having a more severe diagnosis were calculated using a multiple logistic regression model comparing each step on the continuum of disease progression to the one immediately preceding it.

Conclusions: Women who gave birth to at least one child had an increased risk of progressing from CIN 3 to SCC compared to nulliparous women (OR=3.83, 95% CI: 1.60-9.18), but this increase in risk of progression for women who have had at least one child was not seen in the lower grade lesions. No significant association was observed between smoking or oral contraceptive use and disease progression. The null findings suggest that the role of host and viral cofactors along with other environmental cofactors in determining the risk of progression should be further explored. Establishing when cofactors act to increase the risk of progressing to more severe precancerous stages will strengthen our general understanding of the etiology of cervical cancer and provide insight into the behavioural, viral, and host characteristics which may assist primary strategies against this disease.
The human papillomavirus (HPV) minor capsid protein L2 plays a necessary role in infection and is a candidate protective antigen. In contrast to the major capsid protein L1, vaccination against L2 elicits antibodies that neutralize diverse oncogenic HPV types, albeit at a lower titer than the type-restricted antibodies elicited by L1 virus-like particles (VLPs). Notably, L2 vaccines can be produced in bacteria, potentially lowering the cost manufacture as compared to the current L1 VLP vaccines manufactured in yeast or insect cells. A broad spectrum and low cost HPV vaccine is needed to address the critical public health need in developing countries because they bear 80% of the global cervical cancer burden. The licensed L1 VLP-based vaccines are currently unaffordable for sustained and widespread implementation in developing countries. Further, the licensed L1 VLP vaccines target only two of the 15 known oncogenic HPV types, triggering ongoing efforts to develop more highly multivalent formulations. Our objective is to produce an HPV vaccine based on a single antigen, L2, in bacteria that is affordable in low resource countries and offers protection against all oncogenic HPV types.

An epitope RG-1 defined by neutralizing monoclonal antibody RG-1 that is mapped to amino acid residues 17–36 of L2 is recognized by these broadly cross-protective antibodies. Multimeric antigens constituting repeats of defined L2 immunogenic domains, encompassing the RG-1 epitope and other conserved neutralizing epitopes from diverse HPV types were produced in bacteria and explored as candidate pan-oncogenic preventive HPV vaccines. Results indicated that the breadth and titer of cross-neutralizing antibodies can be increased when L2 is presented in a multivalent form. L2 multimers induced robust antibody responses against diverse HPV types including those not directly targeted by the vaccine. Neutralizing antibodies were persistent several months following immunization in both rabbits and mice. Additionally, animals vaccinated with an L2 multimer vaccine adjuvanted with alum were protected from challenge by multiple diverse HPV types.

Complete unit operation for a simple, straightforward, robust and scalable production platform involving fermentation and purification of the broad spectrum HPV L2 vaccine candidate from recombinant bacteria has been developed by Shantha Biotechnics. Alum-based stable formulations that elicit strong immune responses were optimized and shown to provide protection in animal models. The simplicity of manufacturing process, high yields and pre-clinical results from animal models suggest promise to meet the challenge of delivering an affordable vaccine. The progress toward development of an L2-based second generation HPV vaccine will be presented.

HPV pseudoviruses (PsV) composed of the viral L1 and L2 proteins, can encapsidate almost any 6-8 kb target plasmid. They are attractive gene transfer vehicles as infectious titers of up to 10e11 can easily be generated in culture. In a mouse model, we showed that de novo expression after intravaginal instillation of HPV PsV is transient and restricted to cervicovaginal epithelial cells at sites of trauma. Vaginal immunization with HPV PsV encoding respiratory syncytial virus (RSV) M2 as a model antigen induced broad-based systemic immune responses and potent genital CD8+ T cell response in the female genital tract against the antigen. Using MHC class 1 tetramer conjugated with an immunodominant peptide from M2, we tracked M2-specific CD8+ T cells in various tissues including the female genital tract after immunization. At day 8 post secondary immunization, we observed a massive (5 fold) expansion of total CD8+ T cells in mice immunized with HPV-M2 PsV compared to controls. Importantly, the increase in the number of CD8+ T cells was mainly attributable to the expansion of M2-specific CD8+ T cells that represented up to 80% of CD8+ T cells present in the genital tract. Many of the T cells were intraepithelial, rather than submucosal. The magnitude of the genital CD8+ T cell response was far superior to the response observed in draining lymph nodes, spleen and blood. Intravaginal HPV prime/boost immunization, using different HPV types in the prime and boost to overcoming antibody-mediated inhibition of infection, induced 10 times more genital Ag-specific CD8+ T-cells than after priming alone or after intramuscular prime/boost with an Adenovirus 5 vector. A high frequency of cervicovaginal CD8+ T cells with an effector memory phenotype was maintained 100 days after booster immunization. HPV PsV vaccination of the upper respiratory tract also generated a detectable intravaginal T cell response, but it was much lower than that induced by intravaginal vaccination. These data indicate that intravaginal HPV immunization preferentially induces robust CD8+ T cell responses in the genital tract. They may have implication in the design of vaccines against HPV-induced genital neoplasia and other sexually transmitted diseases in which CD8+ T cells may be required for protection.
Human papillomavirus (HPV) has been shown to be associated with several important cancers, including anogenital cancer, and a subset of head and neck cancers. This association has created an opportunity to control these cancers through vaccination against HPV. The existing preventive HPV vaccines are not effective in controlling pre-existing HPV infection. Thus, in order to accelerate the control of HPV-associated malignancies and to treat currently infected patients, it is important to develop therapeutic HPV vaccines. Two HPV oncogenic proteins, E6 and E7, are consistently co-expressed in HPV-associated cancers and are important in the induction and maintenance of cellular transformation. Therefore, immunotherapy that targets E6 and/or E7 proteins may provide an opportunity to treat HPV-associated malignancies. Various forms of therapeutic HPV vaccines, including protein/peptide-based vaccines, viral/bacterial vector-based vaccines, DNA vaccines and cell-based vaccines, are currently being developed in preclinical models, with potential for clinical translation. Among these, DNA vaccines have emerged as an attractive approach for therapeutic HPV vaccine development due to its safety, simplicity and ease of preparation. However, DNA vaccines have limited potency because it lacks the intrinsic ability to amplify and spread like vector-based vaccines. Therefore, it requires the employment of strategies to enhance DNA vaccine potency. Professional antigen-presenting cells, such as dendritic cells (DCs), are the most effective cells for priming antigen-specific T cells. We have thus developed several innovative strategies to enhance DNA vaccine potency by modifying the properties of DCs. The impressive data generated from the preclinical data have led to several HPV DNA vaccine clinical trials. With continued progress in the field of vaccine development, therapeutic HPV vaccines may provide a potentially promising approach for the control of lethal HPV-associated malignancies.

Licensed HPV vaccines, based on virus-like particles (VLP) self-assembled from major capsid protein L1, protect against two (HPV16, HPV18) of the 15 high-risk HPV, yet cross-protect against non-vaccine genital types only at low-level. Conversely, the minor capsid protein L2 contains type-common epitopes that can induce low-titer neutralizing antisera and cross-protection against heterologous papillomavirus types in animal studies. However, L2 is subdominant to the immune system when co-expressed as L1-L2 VLP.

Objectives: To augment immunogenicity of L2 and provide broad cross-protection against heterologous HPV. We have established an HPV16 VLP based vaccine (RG1-VLP) that displays a key cross-neutralization L2 epitope (RG1) inserted into an immunogenic surface loop of HPV16 L1. Display of L2 induces broadly cross-neutralizing antisera to a number of high-risk and low-risk mucosal HPV without compromising type-restricted L1-specific immunity (1).

Methods: Immunization with RG1-VLP using the human applicable adjuvant alum-MPL (similar to ASO4) induced broadly cross-neutralizing antibodies against mucosal high-risk HPV16/18/31/45/52/58, mucosal low-risk HPV6/11 and cutaneous HPV5 in both rabbits and mice. To evaluate the full spectrum of vaccine efficacy, neutralization assays were established based on novel pseudovirions and infectious cutaneous HPV virions isolated from patients’ skin warts. Antisera to RG1-VLP additionally cross-neutralized mucosal high-risk HPV26/33/35/39/56/59/68/73, mucosal low-risk HPV32, and cutaneous HPV2/27/3/76, but not HPV1/4. Using an experimental genital challenge model, passive transfer of RG1-VLP immune serum effectively protected mice against vaginal infection with pseudovirions of phylogenetically divergent mucosal high-risk HPV16/45/73/56/59.

Conclusion: This novel RG1-VLP is a promising vaccine candidate to protect against a broad-spectrum of mucosal and cutaneous HPV infections and diseases.
Persistent infection by high risk genotypes of human papillomavirus (HPV) is the cause of cervical cancer, which remains one of the most common cancers among women worldwide. In addition, there is a growing appreciation that high risk HPVs are associated a number of other cancers including anogenital cancers as well as a subset of head and neck cancers. Recently, prophylactic HPV vaccines targeting the two most prevalent high risk HPVs (HPV16 and HPV18) and have been deployed in large-scale vaccination campaigns. However, the extent to which prophylactic vaccines confer protection against other high risk genotypes is largely unknown and prophylactic vaccines have been shown to be ineffective against pre-existing infection. Thus there continues to be an urgent need for effective therapeutic vaccines against HPV. The E7 protein of HPV16 has been widely studied as a target for therapeutic vaccines in HPV-associated cancer settings because HPV16 is the most prevalent of the high risk HPV genotypes. However, HPV16 accounts for only about 50% of cervical cancers and there are at least 15 other high risk HPVs that are known to be oncogenic. We have developed a novel, broad-spectrum, therapeutic vaccine (Pentarix) directed at the E7 proteins from five of the most prevalent high-risk genotypes of HPV worldwide (HPV16, 18, 31, 45 and 52) that together account for more than 80% of all HPV-associated cancers. Pentarix is a recombinant protein-based vaccine that elicits strong, multi-genotype specific CD8 T cell immunity when administered to mice in combination with adjuvants comprised of agonists of the TLR3 or TLR9 family of innate immune receptors. ELISPOT analyses reveal that Pentarix elicits broad cellular immunity reactive to each of the genotypes contained within the vaccine. Furthermore, large, established E7-expressing TC-1 tumors undergo rapid and complete regression after therapeutic vaccination of mice with Pentarix. Together, these data suggest that Pentarix may have significant clinical value for patients with E7-positive, HPV-associated precancerous lesions or malignant disease.

**SULINDAC HAS ANTI-NEOPLASTIC ACTIVITY TOWARDS CERVICAL CANCER CELLS**


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**Objectives:** Sulindac a commonly used non-steroidal anti-inflammatory drug (NSAID) has been demonstrated to have anti-neoplastic activities. A recent study looked at the activity of this drug on the HPV18 positive cervical cell line, HeLa, established that not only did it induce apoptosis in cells but also a post-transcriptional degradation of the HPV18 oncoprotein, E7. This study aimed to substantiate previous findings and to extend the analysis to other cervical carcinoma cell lines with differing origins, HPV status and viral DNA content. Once the anti-neoplastic activity of the drug was verified, the mechanism of action was interrogated using whole transcriptome microarray analysis.

**Methods:** Three cervical cancer cell lines were examined, the adenocarcinoma derived HPV18 positive HeLa, the squamous cell carcinoma derived HPV16 positive SiHa and the HPV negative cervical cell line C33A. Sulindac had a time and dose dependent growth inhibitory effect on all cell lines. However, the most potent response was observed in the HeLa cells, with the IC50 value approximately 200µM less than the other two cell lines. Further analysis of the HeLa cells demonstrated that this activity occurred predominantly through the induction of apoptosis but additionally by cell cycle arrest. The previous findings that sulindac induced a post-transcriptional degradation of HPV18 viral oncogene E7 were validated. However, in comparison with the findings from the previous study these results were observed with significantly lower concentrations of sulindac, 115µM compared to 500µM. In addition, it was demonstrated that a decrease in COX activity may have a role to play in the anti-proliferative activity of sulindac. To further investigate the molecular mechanism of action of sulindac on the HeLa cells we performed genome-wide microarray analysis.

**Conclusion:** The data indicates that the anti-neoplastic activities of sulindac are multifaceted. Since most cancers progress through the action of multiple pathways, drugs that simultaneously block several pathways might be particularly effective as therapeutic agents. Therefore, these results suggest that NSAIDs may offer potential as novel therapeutics for cervical cancer.
CERVICAL CANCER SCREENING BY SELF-SAMPLING FOR P16 WITH TWO DIFFERENT SAMPLING SYSTEMS

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Objectives: Primary screening by HPV testing has a high sensitivity, however its specificity remains limited. In contrast, p16INK4a is only expressed in the oncogenic process of cervical cancer. The majority of cervical cancer cases are still associated with absent or deficient screening because of inconvenience often associated with obtaining Pap smears. Patient-obtained vaginal sampling for analysis of HPV DNA has a sensitivity of detection of high-grade cervical lesions that is at least as equivalent compared to Pap smear. However, it remains unclear what sampling method is best for self-sampling and what diagnostic assay provides high sensitivity and specificity.

Methods: We compared efficiency and patient handling of two self sampling devices such as vaginal brush and vaginal lavage (Delphi Screener, Delphi Bioscience) sampling. Gynaecological examination including smears for Pap, Cervatec and HPV testing and colposcopic examination with biopsies was performed. All samples were examined by p16 ELISA (Cervatec, mtm laboratories, Heidelberg). This pilot study included 152 patients recruited at our colpo clinic. 66 patients (43%) presented with atypical Pap smear, 69 (46%) were hr-HPV positive and 31 had CIN 2+. Handling of the self sampling devices was rated acceptable or good in 62% of the cases. But only in 2 of 66 atypical pap smears the self sampled p16 ELISA turned out to be positive (3%).

Conclusions: Comparing vaginal sampling with vaginal lavage, we could not find any significant difference in respect to the detection rate for HPV and p16-expression. In conclusion, our results showed that self-sampling followed by p16 ELISA analysis is not suitable to detect CIN.

IMIQUIMOD AND HPV THERAPEUTIC VACCINATION IN PATIENT WITH VULVAL INTRAEPITHELIAL NEOPLASIA.

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Vulval intraepithelial neoplasia (VIN) is a premalignant condition, which is frequently associated with type HPV 16 infection, and multifocal disease has high rates of surgical treatment failure. This study treated the high grade VIN lesions of 19 women topically for 8 weeks with imiquimod (an immunostimulatory cream) followed by three monthly vaccinations with TA-CIN, a fusion of the HPV 16 L2 minor capsid, E6 and E7 oncogenic proteins. The rationale was that the imiquimod would altered the local balance between CD8 T cells, which can destroy the HPV infected premalignant cells, and regulatory T cells which suppress immune activity, with the vaccination boosting the effective HPV 16 oncogene T cell immunity. A majority of women had objective clinical responses and no symptoms one year after receiving the treatment and this therapeutic effect was associated with both increased CD8/Treg ratios locally and boosted E6/E7 T cell responses systemically. The potential for increased vaccine immunogenicity by addition of an adjuvant has been established in preclinical models so a future goal is to test the adjuvanted vaccine in combination with local immune stimulation to further increase patient response rates.
HOW PROPHYLACTIC VACCINES PREVENT HPV INFECTION OF THE FEMALE GENITAL TRACT IN A MURINE MODEL

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We have used our recently described Human papillomavirus (HPV) pseudovirus (PsV) cervicovaginal murine challenge model to study vaccine-induced protection from HPV infection. We compared vaccination with HPV virus-like particles (VLP) composed of the L1 major capsid protein, the composition of the current commercial HPV vaccines, to a candidate L2 polypeptide vaccine comprised of the highly conserved N-terminal amino acids of the L2 minor capsid protein. L1 VLPs contain the immunodominant neutralization epitopes, which are type-restricted, while the L2 polypeptide induces broadly cross-neutralizing antibodies directed against L2 epitopes that aren’t exposed on free virions.

We have determined that HPV PsV infection requires transient disruption of epithelial integrity, as the first step in infection is PsV binding to the basement membrane (BM) at the dermal-epidermal interface. In a process that takes several hours, the PsV undergoes a required conformational change on the BM that exposes the cryptic L2 cross-neutralization epitopes, followed by transfer to the keratinocyte surface, where the PsV is internalize, resulting in infection.

In the murine model, parenteral immunization with HPV16 L1 VLPs induces type-restricted protection similar to that seen in human clinical trials, while parenteral L2 immunization induces protection against both homologous and heterologous PsV challenge. In VLP vaccinated mice, homologous, but not heterologous, PsV fails to bind the BM or the keratinocytes and the cryptic L2 epitopes are not exposed. Similarly, systemic passive transfer of immune serum from L1 VLP vaccinated women or rabbits conferred type-restricted protection in the murine model. High doses of passively transferred sera also prevent BM binding and keratinocyte binding of homologous PsV whereas low doses allowed BM binding but prevented infection by preventing transfer to the surface of the keratinocytes. Regardless of the concentration, L2 vaccine-induced antibodies allow BM association, but prevented association with the cell surface. Thus L2 antibodies appear to prevent in vivo infection at one of the two distinct steps inhibited by L1 antibodies. These results provide mechanistic insights into the remarkable, but type-restricted, efficacy of L1 VLP vaccines and encourage further development and clinical testing of broadly cross-neutralizing L2-based vaccines.

COMPARISON OF IMMUNOGENICITY OF TWO PROPHYLACTIC HUMAN PAPILLOMAVIRUS (HPV) VACCINES AT MONTH 36

Einstein MH on behalf of the HPV-010 Study Group

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Objectives: Vaccine-induced protection against HPV-16 and -18 has been demonstrated for HPV-16/18 AS04-adjuvanted vaccine (HPV-16/18 vaccine; GlaxoSmithKline Biologicals) and HPV-6/11/16/18 AAHS-adjuvanted vaccine (HPV-6/11/16/18 vaccine; Merck). In a comparative trial in women 18–45 years (NCT00423046), geometric mean titres (GMTs) of serum neutralising antibodies (nAbs) at Month 7 (1 month after last vaccine dose) were higher with HPV-16/18 vaccine vs HPV-6/11/16/18 vaccine for HPV-16 (80% vs 40%, p=0.03) and was 55% vs 29% (p=0.19) for HPV-18; GMTs were comparable between groups for the two types. Contrary to previous timepoints, the memory B-cell response to HPV-18 was no longer higher with HPV-16/18 vaccine at Month 36, possibly due to reduced sample size. The clinical impact of differences in immune response between the vaccines (through Month 36) on long-term protection remains to be clarified; follow-up of this cohort is still ongoing.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>GMT Serum nAb (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td>HPV-16/18 vaccine</td>
</tr>
<tr>
<td>HPV-16</td>
<td></td>
</tr>
<tr>
<td>18–26</td>
<td>3845 (2804–5272)</td>
</tr>
<tr>
<td>27–35</td>
<td>1898 (1419–2538)</td>
</tr>
<tr>
<td>36–45</td>
<td>1794 (1278–2519)</td>
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<tr>
<td>HPV-18</td>
<td></td>
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<tr>
<td>18–26</td>
<td>1594 (1177–2158)</td>
</tr>
<tr>
<td>27–35</td>
<td>943 (713–1247)</td>
</tr>
<tr>
<td>36–45</td>
<td>904 (625–1306)</td>
</tr>
</tbody>
</table>

* Superiority demonstrated in total vaccinated cohort (p<0.001) for HPV-16 and HPV-18

The proportion of antigen-specific CD4+ T-cell responders (subjects with ≥500 cells expressing ≥2 cytokines/million cells) was higher with HPV-16/18 vaccine vs HPV-6/11/16/18 vaccine for HPV-16 (80% vs 40%, p=0.03) and was 55% vs 29% (p=0.19) for HPV-18; GMTs were comparable between groups for the two types. Contrary to previous timepoints, the memory B-cell response to HPV-18 was no longer higher with HPV-16/18 vaccine at Month 36, possibly due to reduced sample size. The clinical impact of differences in immune response between the vaccines (through Month 36) on long-term protection remains to be clarified; follow-up of this cohort is still ongoing.
HPV is a localized infection and it is not until the cells reach maturation that the late proteins L1 and L2 are expressed. Here the viral DNA is packaged within the viral capsid made up of L1 and L2. When the mature squamous epithelial cell normally desquamates, infectious virus is released. Consequently, this process does not involve cell death and results in few triggers for a systemic immune response. Consequently, local immune responses are likely critical in initial viral control. The natural history of HPV and SIL show two patterns: rapid clearance within 4-8 months and slower clearance within 2-3 years. The former is likely associated with innate responses such as expression of Toll-like receptors (TLR). HPV 16 has been shown to down regulate TLR expression in both in vitro and in vivo studies. Other HPV types do not appear to have the same abilities. TLR expression also has the ability to influence the development of cell-mediated immune responses. We recruited 95 young women with CIN 2 into a longitudinal study. We found that approximately 70% of these young women cleared their CIN 2 spontaneously. This however was type dependent in that only 55% of HPV 16 associated CIN 2 cleared. In this cohort, we examined the role of TLR receptors, systemic immune responses and clearance of CIN 2. In this study, we found a trend association between TLR expression and CIN 2 clearance but this was much weaker than in women who had HPV 16 but no CIN 2. This suggests that TLR receptors are more important early on in HPV’s natural history. We also examined systemic immune responses to HPV 16 E6 and E7. Local immune responses can be difficult to interpret since several factors appear to affect this milieu including oral contraceptives, smoking and the predominant epithelial type. For example, we found that cytokines such as ILs- alpha and -beta, ILs -6 and -8 and MIP-1 alpha were 10-100 fold higher among sample obtained from women with large areas of ectopy compared to those with predominantly mature squamous epithelium. In another study we found that squamous metaplasia but not ectopy alone predicted acquisition of HPV 16 infections. Expression of TLR receptors may be greater in areas of columnar epithelium. Some of these findings may help explain why young women are vulnerable to HPV infections and yet are able to rapidly clear HPV and SIL.

**Antibody Response to the Quadrivalent HPV Vaccine: Correlation of Total IgG Assay with Pseudovirion Based Neutralization Assay**

Darron R. Brown for the GARDASIL Study Team

**Introduction:** A concordance study was conducted to compare serologic response to the quadrivalent HPV L1 virus-like particle (VLP) vaccine in three different assays: the 4-HPV type competitive Luminex Immunoassay (cLIA), the 9-HPV type Total IgG Luminex assay (Total IgG), and the Pseudovirion Based Neutralization Assay (PBNA). A secondary goal of the study was to potentially broaden the number and type of serological assays available for anti-HPV detection. The comparisons among assays were restricted to HPV types 16 and 18.

**Methods:** For each HPV type, samples were randomly selected across a range of antibody concentrations to provide for comparable representation from different populations (women 23 to 45 years of age and men 16 to 27 years of age) and sampling intervals. A total of 648 samples were selected for evaluating anti-HPV-16 response in the cLIA, IgG-LIA, and HPV-16 PBNA, and a total of 623 samples were selected for evaluating anti-HPV-18 response in the same assays. Separate analyses were performed for HPV-16 and HPV-18. All quantitative comparisons were performed on the log-transformed data and were limited to the subset of sera having a quantifiable result in the assays being compared. The functional relationship between pairs of assay methods (cLIA, IgG, and PBNA) was estimated separately for each HPV type, by study protocol, and sampling interval using the linear statistical relationship model of Tan and Iglewicz. The Pearson correlation coefficient was also calculated for each protocol and sampling interval sub-grouping.

**Results:** For both HPV-16 and HPV-18, for all samples tested, the cLIA and IgG assays were strongly associated, and the PBNA was strongly associated with both the cLIA and IgG assays. For all samples combined, the correlation coefficients computed on the log transformed data were very high across the three assays and two HPV types. Additionally, the estimates of slope from the fitted linear statistical relationship model were all near unity, providing further evidence of strong agreement between assays.

**Conclusion:** The cLIA, total IgG assay and PBNA are very highly correlated and reflect measurement of neutralizing antibody against HPV types 16 and 18 L1 VLPs.
LONG TERM EX VIVO MONITORING OF MEMORY CD4 T HELPER CELL RESPONSES IN WOMEN IMMUNIZED WITH GARDASIL® OR CERVARIX™

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OBJECTIVES: The virus-like-particle (VLP)-based quadrivalent AAHS-adjuvanted vaccine Gardasil® and the bivalent AS04-adjuvanted vaccine Cervarix™ provide prophylactic protection against infections with the human papilloma virus (HPV) types 6/11/16/18 and 16/18, respectively. Serological data show differences in titre and persistence of specific antibodies yet clinical efficacy for cervical dysplasia by vaccine HPV types is comparable.

CD4 T cellular immune responses are important for induction and maintenance of humoral responses. We conducted a non-randomized, cross-sectional study including 34 subjects, immunized with either Gardasil® or Cervarix™ on average 4.5 years prior to study enrolment. We assessed CD4 T cellular immune responses, including the cross-reactive vaccine-induced cellular immune responses to heterologous HPV-types (HPV31/45) by an ex vivo test.

METHODS: One ml of whole blood was stimulated with HPV-L1 or HPV-E6/E7 synthetic peptide pools for 14-20 hours. Memory CD4 T cells were identified by intracellular staining for CD4, CD154, IL-2 and IFN-gamma and subsequently analysed by flow cytometry.

RESULTS: Mean frequencies of L1-specific CD4+/CD154+/IL-2+ T cells in Gardasil® vs. Cervarix™ vaccinees were for HPV6: 0.045% vs. 0.045%; HPV11: 0.051% vs. 0.033%; HPV16: 0.053% vs. 0.058%; HPV18: 0.027% vs. 0.037%; HPV31: 0.030% vs. 0.016%; HPV45: 0.015% vs. 0.036%. Generally, differences were not statistically different. Of note, also T cell cross reactivity with low risk HPV6/11 was seen in Cervarix™ vaccinees.

CONCLUSIONS: High frequencies of memory CD4 T helper cells specific to HPV L1 antigens are detected up to 6 years after vaccination with either Gardasil® or Cervarix™ at comparable levels to those detected 6 months after the third immunization. Direct comparison showed almost no significant differences between Gardasil® or Cervarix™ There is a trend for Gardasil inducing stronger T cell responses to low-risk HPV types, whereas Cervarix to high-risk HPV types. On the polyclonal T cell level there is a strong cross reactivity with non-vaccine types.

AGE-SPECIFIC HUMAN PAPILLOMAVIRUS ANTIBODY AND CONCORDANT DNA PREVALENCE: A GLOBAL REVIEW

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Objectives: Worldwide cumulative HPV exposure is difficult to fully understand due to the large number of studies measuring HPV in different populations using various techniques. In this study, we aimed to compile and summarize global age-specific HPV serological and DNA prevalence in order to analyze trends pertinent to the development of HPV prophylactic vaccination strategies worldwide.

Methods: We conducted a systematic review of articles in Medline examining cross-sectional HPV antibody and concordant DNA prevalence for HPV types 16, 18, 6, and 11. Non-high-risk male and female populations with age-specific data were included from 123 studies.

Conclusions: Most populations were from Europe (37%) and North America (26%), followed by Asia and Australia (19%), Latin America (8%), and Africa (6%). Ages of included populations ranged from a few hours to over 90 years. HPV 16 antibody prevalence was generally higher in Africa, Latin America, and North America, more prevalent among women than men, and peaked around age 25-40 years in women throughout the world. HPV 18 seroprevalence was generally lower than HPV 16, with a relatively later peak in age. HPV 6 and 11 both peaked later in age than either HPV 16 or 18. In females aged 9-26 years, for whom the HPV vaccine is currently approved in the United States, HPV 16 seroprevalence ranged from 0-43% worldwide. Among studies with concordant HPV and serology data, HPV 16 and 18 DNA prevalence peaked 10-15 years before antibody prevalence. Associations between HPV 16 and 18 antibody and DNA prevalence were unclear in populations with age-specific concordant data, suggesting HPV serology is not a straightforward indicator of past HPV infection. Few serology studies and no concordant DNA/serology studies presented data on females younger than 15 years of age; more data are needed worldwide for this target age group for HPV vaccination.
DEVELOPMENT AND QUALIFICATION OF AN AUTOMATED PSEUDOVIRION-BASED HPV-NEUTRALIZATION ASSAY

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1 DKFZ-EMBL Chemical Biology Core Facility and 2 German Cancer Research Center (DKFZ), Heidelberg, Germany; 3 Merck & Co., Inc. New Jersey, USA

Objectives: To broaden the number and type of serological assays available for anti-HPV detection the makers of the quadrivalent HPV vaccine Merck planned to conduct a large concordance study of three HPV serology assays, the HPV-4 competitive Luminex Immunoassay (cLIA), the HPV-9 Total IgG Luminex assay (IgG), and a new Pseudovirion Based Neutralization Assay (DKFZ PBNA) for HPV 16 and 18 developed by DKFZ. In two qualification studies the performance characteristics of the DKFZ PBNA and its correlation to the cLIA and IgG assays were assessed.

Methods: The DKFZ PBNA uses genetically modified HeLa cells as infection target for HPV Pseudovirions delivering secreted Gaussia Luciferase as reporter gene. Its automated add-on assay format enables testing of 22 sera in 10 concentrations per 384-well plate for neutralizing antibodies, the separation of assay plate production and assay performance and is therefore well suited for high-throughput screening. For each HPV type 44 distinct samples taken from completed Gardasil trials and representing different levels of antibody responses and a reference standard were analyzed. All pair-wise comparisons were made among the three assay methods and functional relationships were estimated using the linear statistical relationship model and Pearson correlation coefficient. The PBNA data was also assessed for reproducibility both within and between qualification studies.

Conclusions: For both HPV-16 and HPV-18, there was excellent agreement between the PBNA and the cLIA (correlation coefficients were very high and slope coefficients were near unity). The agreement between the PBNA and cLIA was comparable to that seen between the newly generated and original results generated within the cLIA. Throughout the response range, PBNA titers (reciprocal of the mid-point dilution of the dilution-response curve) were, on average approximately 5-times the cLIA antibody concentration (reported in mMU/mL) for HPV-16 and HPV-18, respectively. For both HPV-16 and HPV-18, the agreement between the PBNA and IgG was comparable to that seen between the cLIA and IgG assays. The results of the qualification experiments supported the initiation of the large-scale concordance study comparing the cLIA, IgG and PBNA assays for HPV-16 and HPV-18.

GENE EXPRESSION PROFILING ON CERVICAL SWABS FOR MOLECULAR CLASSIFICATION OF INTRAEPITHELIAL NEOPLASIA.

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Objective: With the current histopathological classification, the evolution of cervical intraepithelial neoplasia (CIN) still remains uncertain while even the high grade lesions (CIN3) are able to spontaneously regress. We propose a molecular classification for cervical dysplasia based on genomic signatures of cervical cells from swabs.

Methods: We studied 71 endocervical samples from 53 women presenting the three grades of CIN (26 CIN1, 13 CIN 2, 14 CIN 3) and 18 controls, using a pangenomic 135K gene expression microarray. Cells were collected by a non-invasive method using cytobrush before RNA extraction. We identified 4 clusters of gene expression corresponding to 3 genomic signatures. The first cluster was significantly associated to CIN 1, regrouping genes involved in the regulation of immune response. The second cluster was overexpressed in CIN3 with genes intervening in cellular metabolism. The two latest clusters group samples from different CIN classes and were associated to keratinocyte differentiation and viral RNA signature.

Conclusion: Based on the three biological signatures identified we could now propose a molecular classification of CIN. A future study will evaluate some biomarkers from these 3 clusters for their diagnosis and prognosis relevance.
THE ROLE OF CHROMOSOMAL INTEGRATION TO INDUCE THE TRANSFORMING TYPE OF HPV GENE EXPRESSION IN CERVICAL LESIONS

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1 Dept of Microbiology, 2 Dept of Pharmacology, 3 Dept of Obstetrics and Gynaecology, 4 Dept of Pathology, Fac of Medicine, Khon Kaen University, Khon Kaen, Thailand. 5Dept of Applied Tumor Biology, Institute of Pathology, University of Heidelberg, Heidelberg, Germany

Background: Upregulation of the E6-E7 oncogenes of high-risk human papillomaviruses (HR-HPVs) in basal squamous epithelial cells marks the shift from permissive to transforming HR-HPV-infections. Integration was hypothesized to trigger the deregulated oncogenes expression pattern and relative degree of chromosomal instability conferred by distinct HR-HPV types may be substantially different.

Methods: The physical status of HR-HPV genomes was analyzed in 99 biopsies of squamous epithelial lesions induced by HPV types 16, 18, 31, 33, 45 and 58 using the amplification of papillomavirus oncogene transcripts (APOT) assay. p16 INK4a immunohistochemistry was used to identify lesions with the transforming mode of HPV gene expression.

Results: In all normal and CIN 1 (n=35) samples no integrated HPV genomes were detected, however, they were found in 7.9% (3/38) of the CIN2+ samples and 53.8% (14/26) of the invasive carcinomas. The frequency of integrated HR-HPV genomes showed marked differences for individual HR-HPV types. HPV 16, 18 and 45 were found substantially more often to be integrated as compared to HPV types 58, 31 and 33. In p16 INK4a positive samples, integrate derived transcripts were detected in 5% of CIN2+ and 50% of carcinoma samples. Episome derived transcripts were detected in all p16 INK4a negative samples.

Conclusions: These data further confirm the hypothesis that the shift to the transforming mode of HPV-gene expression indicated by p16 INK4a overexpression substantially precedes the emergence of squamous epithelial cell clones with chromosomally integrated viral genome copies in the progression cascade of HPV-induced preneoplastic lesions. Individual HR-HPV types confer a substantially different degree of transforming power and chromosomal instability to their host cells reflected by the relative frequency of integration in advanced lesions.

ANTIGEN EXPRESSION AND LOCALIZATION FROM A MVA-BASED VECTOR (RG3484) ENCODING GENES FOR HUMAN IL-2 AND MODIFIED HPV16 E6 AND E7

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Objectives: HPV-associated neoplasia of the cervix represent an excellent target for therapeutic vaccines because the tumor-associated non-structural viral antigens are clearly defined; HPV E6 and E7 oncoproteins are exclusively expressed in HPV-infected, pre-neoplastic and neoplastic cells. The MVA-based RG3484 viral vector expresses human IL-2 and modified HPV16 E6 and E7 proteins with deletions in the binding domains for pRB and p53. We assessed subcellular localization of RG3484 expression products in cell cultures and cellular location in murine and human skin samples after subcutaneous (sc) injection.

Methods: NIH-3T3 murine fibroblasts were infected with RG3484, then stained with E6, E7, and MVA monoclonal antibodies and co-stained to assess subcellular antigen localization by confocal microscopy 24 hours later. Full-thickness skin was collected from mice 16 hours after single sc injection of RG3484 (10^7 pfu) to assess E6, E7, and MVA protein expression. As part of a phase 1 trial, 10 healthy volunteers each received a single sc injection of 5x10^7 pfu RG3484 or placebo in the thigh. Skin biopsies taken 2-10 days later were assessed for E6 and E7 protein expression colocalized with dendritic cell markers compared with control biopsies from the opposite thigh. E6 mRNA expression in biopsies was assessed by qRT-PCR.

Results: E7 and E6 proteins were expressed in the endoplasmic reticulum (ER) and early endosomes of RG3484-infected NIH 3T3 cells, whereas MVA proteins were expressed in ER and late endosomes. In mice, MVA and HPV16 E7 protein were expressed in necrotic areas of sc tissue and adipocytes; E6 protein was not detected due to high background staining. Skin biopsies from subjects in the phase I trial showed an inflammatory infiltrate at the RG3484 injection site and strong expression of E7 in human dendritic cells for up to 10 days after injection; expression of E6 mRNA was confirmed by qRT-PCR in skin samples.

Conclusion: These results indicate appropriate expression of RG3484 antigens in mouse and human skin following sc injection; assessment of immune response to RG3484 in human volunteers is ongoing.
AN E6 PROTEIN BASED RAPID DIAGNOSTIC TEST FOR CERVICAL PRE-CANCER AND CANCER
– DETECTION OF E6 ONCOPROTEIN FROM FIXED CELLS

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3 Johns Hopkins Bloomberg School of Public Health, Baltimore, USA
4 American Society for Clinical Pathology Institute, Washington, USA

Cervical cancer is the major cause of cancer related mortality of women living in developing countries, causing over 250,000 deaths / year worldwide. Implementation of appropriate technology for detection of cervical pre-cancer and cancer in low-resource settings constitutes a key element towards reduction of this unacceptably high death toll.

Arbor Vita Corporation (AVC), in collaboration with the Program for Appropriate Technology in Health (PATH), has developed the AV Avantage HPV E6 Test, a simple lateral flow based test for detection of the E6 oncoprotein of the three most prevalent HPV types in cervical cancer, HPV 16, 18, and 45, in cervical exfoliated specimens. Elevated expression of E6 (and E7) oncoprotein is thought to be a prerequisite for development of high-grade cervical neoplasia, and a pre-clinical pilot study suggests that the AV Avantage HPV E6 Test specifically detects women with high-grade lesions and cancer rather than HPV positive women with low grade lesions or no pathology1. The test is currently being evaluated in a large clinical study in China.

Ongoing activities focus on integrating additional HPV types into the AV Avantage HPV E6 Test to cover the 8 HPV types most prevalent in cervical cancer (HPV 16, 18, 31, 33, 35, 45, 52, 58), and to further simplify the workflow. The current test format does not require complex machinery, but further simplification of the workflow and improved test versatility appears feasible, for example via the use of cervical specimens stored in customary cervical swab specimen fixatives. Data on application of the AV Avantage HPV E6 Test for detection of E6 oncoprotein from fixed cells and progress on the expansion towards additional HPV types will be presented.


SIGNIFICANCE OF MOLECULAR PATHOLOGICAL EXAMINATION OF CERVICAL BIOPSIES FOR HIGH RISK GROUPS WITH HPV ASSOCIATED CERVICAL DYSPLASIA

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Objectives: To evaluate the significance of molecular pathological examination of biopsy samples with HPV induced cervical lesions, especially for young women, who are all too frequently affected by overtreatment. Therefore we performed a fluorescence in situ hybridisation (FISH) based test.

Methods: The preliminary results of ten biopsy samples of ten young women, aged between 21 and 24 were extracted from a study of 105 patients and grouped according to an initial histological diagnosis. They were tested for their type of HPV infection by PCR-ELISA and for p16 expression by immunohistochemistry. Furthermore we carried out the Abbott/Vysis FISH test to detect HPV genomic integration and the amplification status of MYC and TERC on formalin fixed biopsy samples.

Results: Among ten patients with an average age of 23.2, there were one CIN III, four CIN II, four CIN I lesions, and one case showed no dysplasia. HPV infection was detectable in all cases with CIN II/CIN I, and in one case with CIN I. The p16 staining revealed consistently high positivity in the CINIII/CINII group. Only two of four CIN I cases showed moderate positivity. The HPV positive CIN I case and the one without dysplasia remained only lightly p16 positive. FISH analysis showed the following results: in all of the CINIII/CINII samples integrated HPV genomes were found, while the other cases displayed no HPV genomic signals. Two of four CIN II lesions were TERC and MYC amplificated whereas no amplification was found in CIN I and benign cases.

Conclusions: Malignant transformation of the cervical surface, measured by HPV integration and TERC/MYC amplification was observed already in a majority of CIN II lesions. However to validate this FISH test especially at high-risk groups it should be integrated in further clinical studies. In this context we are planning a clinical study in young women and pregnant women with HPV infection. By follow-up analysis the reversible changes of cervical dysplasia will be monitored.
HIGH RISK HUMAN PAPILLOMAVIRUS INDUCED EXPRESSION OF ENDOTHelial AND INDUCIBLE NITRIC OXIDE SYNTHASES IN HUMAN UTERINE CERVIX

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Objective: Levels of nitric oxide metabolites are elevated in the cervical fluid of women with high risk human papillomavirus (hrHPV). To elucidate the origin of this elevation we studied the expression of endothelial and inducible nitric oxide synthases (eNOS, iNOS) in the uterine cervixes of 86 women with (n=41) and without (n=45) hrHPV infection. Furthermore, we studied the localization of eNOS and iNOS in various cervical cells in 32 women.

Methods: The expression of eNOS and iNOS, assessed by Western blotting [in mean (95% CI) density units relative to actin] was higher in women with hrHPV vs. those without [eNOS: 33.8 (22.5-45.1) vs 20.2 (6.1-34.3), p = 0.007], [iNOS: 12.0 (7.1-16.9) vs 5.6 (2.0-9.2), p = 0.003]. In hrHPV infected smokers expression of eNOS (p = 0.001) and iNOS (p = 0.008) was reduced vs. nonsmokers. Localization of eNOS and iNOS was studied by immunohistochemistry. Endothelial NOS was found in the vascular endothelium while iNOS was present in the basal squamous epithelial cells. Low grade histological lesions were accompanied with elevated expression of both eNOS and iNOS.

Conclusions: High risk HPV is accompanied with elevated expression of both eNOS and iNOS in the human uterine cervix. Further studies are needed to clarify the clinical significance of these findings.

ASSOCIATION OF INTERLEUKIN-10 GENE PROMOTER POLYMORPHISMS AND GENE EXPRESSION IN HUMAN PAPILLOMAVIRUS CERVICAL LESIONS

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Objectives: An immunosuppressive state had been identified in women with HPV persistence, characterized by high levels of IL-10; this may allow the cervical lesion development. Thus, we determined whether polymorphisms of IL-10 promoter might be associated with an increased risk of cervical lesion and whether the systemic IL-10 mRNA expression level (SIREL) might be influenced by the genotypes in Mexican women.

Methods: Peripheral blood samples from patients with squamous intraepithelial cervical lesions (SICL, n = 171) and non-lesions controls (NLC, n = 166) were used to detect four biallelic IL-10 promoter polymorphisms at -592, -819, -1082 and -1352 sites by allelic discrimination with Taqman probes. The SIREL and in cervix were measured with real time PCR. Cervical swab from women NLC and biopsy specimen with SICLs were used for HPV typing and to evaluate IL-10 RNA expression level. Genotypic and allelic frequencies of the four selected SNPs were evaluated in both groups; we evaluated the SIREL according to the genotypes, and analyzed the association of these polymorphisms with the risk of CL by logistic regression.

Conclusions: Significant differences were not found between genotype frequencies at locus (-819, -1082 and -1352). Individuals carriers of heterozygous IL-10 -592 C/A polymorphism were twice greater odds of having SICL (OR of 2.08 (95% CI, 1.054-4.106), p=0.03, as compared with NCL. The IL-10 expression was significantly higher both at SIREL and in the cervix (p=0.00), however none of the polymorphisms was statistically associated with the IL-10 expression level. The -592 C/A polymorphism of the IL-10 promoter appear to be associated with HPV lesion risk in Mexican women, furthermore, the SIREL and in cervix reported here, increases as the lesion progresses.
**SS 20-8**

**GAIN-OF-FUNCTION WNT-INDEPENDENT ACTIVITY OF THE PYGOPUS2 TRANSCRIPTION FACTOR IN GYNECOLOGIC CANCER**

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**Objectives:** The Pygopus2 (Pygo2) protein was originally described in embryonic development as a core component of the Wnt/β-catenin transcriptional activation complex but we previously found that it has Wnt-independent requirements for the growth of epithelial ovarian cancer. The aim of this study was to uncover novel functions of Pygo2 in gynecologic cancer by determining the proteins with which its N-terminal (Wnt-independent) homology domain interacts.

**Methods:** Glutathione-S-transferase (GST) fusion proteins containing either the N-homology domain or Plant Homology Domain (PHD) of Pygopus were transfected into SKOV-3 (EOC) and HeLa (Cervical) cancer cells. Complexes associating with the fusion protein were purified on glutathione beads and separated on SDS PAGE. Protein bands specific to the NHD but absent from the PHD containing complexes were analyzed by tandem Mass Spectrometry. Proteins were analyzed by in vitro and in vivo protein interaction pulldown assays, immunoblot (western) and immunofluorescence (IF) analysis.

**Conclusions:** In both EOC and cervical cells, the Wnt-independent NHD of Pygo interacts strongly with the product of the TCOF1 gene in its C-terminal region. Using cell fractionation and IF, both Pygo2 and TCOF1 colocalized to the same subcellular region in both SKOV-3 and HeLa cells. Our results indicate that in both epithelial ovarian and cervical cancer, the Wnt-independent function of Pygo2 requires an interaction with the TCOF1 protein.

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**SS 20-9**

**DEVELOPMENT OF A NEW HPV DIAGNOSTIC TOOL BASED ON THE USE OF ARRAYED AND NANOPARTICLE-BOUND DNA PROBES**


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**Objectives:** The EU Project NANO-MUBIOP (www.nanomubiop.eu) aims to develop a new diagnostic tool to detect HPV DNA beyond existing PCR-based methods. The system uses dual hybridization of HPV DNA with a generic probe (which binds multiple HPV types) and a probe specific for each type. The generic probe is attached to nanoparticles (NPs) while specific probes are arrayed on a solid support. An optical detection system will detect binding of NP-targets to arrayed probes. To develop this diagnostic tool, DNA probes specific for each individual 51 HPV types were designed: each type-specific probe contained at least 20% of mismatches with all other types. A set of 9 generic probes was also created and tested in vitro. The efficiency of three-layer NP hybridization and several system parameters were investigated.

**Methods:** i) A database of 147 different HPV genomes was created; 20-base-long probes containing more than 4 mismatches with all non-target DNA were selected taking secondary structures and Tm into account. ii) 5’-biotinylated probes were hybridized with HPV DNA on a nylon membrane by Dot blotting, hybridization was detected using streptavidin-AP and a chemiluminescent substrate. iii) Specific probe/target/generic probe hybridization was tested in plastic wells to which linker-bound, 5’ NH₂-specific probes were attached. Generic probes were radiolabeled and attached to COOH-coated silica NPs. Hybridization was tested using both the free generic probe and NP-bound probes.

**Conclusions:** Dot blot assays revealed that probes specifically hybridize with target HPV DNA and not with human or non-target viral DNA. In multi-well tests the presence of a poly-A spacer arm dramatically increases the rate of hybridization. NPs with a diameter of 105, 120 and 190 nm bind 200 to 400 fmol of generic probe per μg, with 120-nm NPs best suited for detection. The efficiency of the process improves when the probe:target ratio is increased. A bio-probe concentration of 7.5 nM (375 fmol/test) is sufficient for detection. A 10000:1 probe:target molar ratio permits detection of about 15% of HPV DNA molecules under the tested conditions.
ANAL CANCER RISK

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Background: Though traditionally a disease of older women, invasive anal squamous cell carcinoma (ASCC) is now increasing in men. Immune compromised individuals are at greatest risk especially those with HIV infection.

Discussion: Rates of cervical cancer before routine cytology screening were approximately 40-50/100,000 and routine screening in the US has brought the rate down to 8.4/100,000. It is estimated that the ASCC rate for HIV-negative men who have sex with men (MSM) is 35/100,000 with rates in HIV-infected MSM more than twice that. Use of antiretroviral therapy (ART) in HIV infected individuals has not decreased ASCC rates. Cervical cancer rates, however, have decreased in the ART era probably secondary to aggressive screening and treatment of these HIV-infected women. Progression to HGAIN seems to occur with time and at greater rates in older patients and those with lower CD4 counts. There is data to suggest that HIV infected patients can clear anal HPV but that clearance can take 3 years. There is still lack of knowledge of anal cancer among MSM and that hampers screening and potential early detection. Local cellular factors including depletion of CD1a and CD3 cells may predispose HIV infected individuals to progression to HGAIN. Increased rates of HPV 16 integration over time since HIV infection could also promote development of HGAIN. Anal HPV infection is also a risk factor for HIV acquisition. Quadrivalent HPV vaccine may be beneficial in HIV infected individuals.

Conclusion: Multiple factors including cellular changes, decreased screening and poor knowledge about risk seem to affect anal cancer risk in HIV infected individuals. ART does not seem to have made reducing anal cancer rates.

ROLE OF HPV IN HIV TRANSMISSION

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Within the past 2 years, several independent studies have reported increased risk of HIV acquisition among HPV positive men and women. Anal (1), penile (2), and urethral (3) HPV has been shown to be associated with HIV acquisition in men, and the risk appears to increase with the number of multiple HPV genotypes detected. In women, both multiple infection and clearance of cervical HPV have been shown to be associated with HIV acquisition after adjustment for STI co-infection and sexual risk behaviors of the women and their male sex partners (4, 5). The specificity of the association between HPV clearance and HIV acquisition is unlikely to be explained as confounding by shared sexual risk, suggesting the possibility of a true biological cause. Lymphocytic epithelial infiltrates in resolving HPV infections could result in a transient increased susceptibility to HIV by increasing the availability of target cells. However, studies to date are unable to determine if HPV clearance preceded HIV acquisition, or vice versa. It is also conceivable that the pro-inflammatory environment induced by HIV engagement of TLR7 could induce or accelerate the immune response to HPV infection. These recent studies of HPV infection during acute HIV infection raise important questions about the nature of these genital tract co-infections and their impact on ano-genital immune responses.

(1) Chin-Hong, et al. AIDS 2009;23:1135,
(2) Auvert, et al. JAIDS 2010; 53:111,
(3) Smith, et al. JID 2010;201:1677,
(4) Averbach, et al. AIDS 2010;24:1035,
HPV SCREEN AND TREAT IN HIV INFECTED WOMEN
DENNY L A

It is now well documented that women infected with HIV have a higher prevalence and incidence of infection with HPV, infection with high risk types, persistent infection and more rapid progression to cervical dysplasia and cervical cancer. For instance, in a prospective study of 400 HIV positive South African over 36 months [1], 68% of women were high-risk HPV positive at entry and 35% had LSIL cytology and 13% HSIL cytology. Abnormal cytology and high risk HPV infection were strongly correlated with low CD 4 counts and high HIV viral loads. The most prevalent types of HPV were HPV 16, 52, 53, 35 and 18. Incident infection occurred in 22% of women over 36 months and of those infected 94% persisted over an 18 month period, with only 6% clearing infection. The majority of this cohort only started anti-retroviral therapy (ART) 2 years into the study, and at 6 years of follow up, the incidence rate of HPV DNA infection and LSIL cytology had not changed post use of ART, despite improved CD 4 counts. In another South African study [2] in which 6555 women aged 35 – 65 were randomized to two screen and treat strategies, 956 women were found to be HIV positive. HIV positive women had higher rates of HSIL (15% versus 5% in HIV negative women). HPV DNA testing followed by treatment with cryotherapy significantly reduced cervical cancer precursors at 36 months in both HIV negative and HIV positive women. Furthermore complications related to cryotherapy were not different in the HIV positive vs negative groups.

2] Kuhn et al. AIDS 2010;24:2553- 2561

POTENTIAL/RATIONAL OF HPV VACCINE
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HPV and HIV interact in many ways. First, HPV infections are associated with heightened rates of HIV acquisition. This association is described in heterosexual women, heterosexual men and men having sex with men. This association was also seen with both high risk and low risk HPV infections. Resolution of HPV infection was also associated with higher HIV acquisition rate. The rate of HIV acquisition was higher with resolution of LR HPV compared to HR HPV. The presence of external genital warts was also associated with an increased HIV acquisition rate. Second, HIV is associated with higher prevalence and persistence rates of HPV infections in both heterosexual and MSM populations. HIV heightens the risk of HPV related cancers such as anal, cervical, oro pharyngeal, penile, vaginal and vulvar cancers. HIV does increase rates of cancers with increasing immune suppression. For example anal cancer rates may be double in the HIV+ compared to HIV persons. The use of highly active antiretroviral therapy does not seem to alter the risk of cancer in people with HIV/AIDS (PHAs). Third, prophylactic HPV prophylactic vaccines, especially if given prior to sexual debut, may help prevent HIV acquisition later in life by decreasing HPV infections and lesions. It may also better prevent HPV related cancers and external genital warts if given prior to the acquisition of HIV. PHAs may develop less robust and shorter immune response and protection of lesions after the being given of HPV prophylactic vaccine. In preliminary results the quad HPV prophylactic vaccine has shown very good seroconversion rates and adverse events were comparable to the HIV- population. If shown effective in preventing persistent infections or lesions, HPV prophylactic vaccines should be given at the highest level of CD4 count. PHAs may benefit at different rates of the prophylactic vaccines: for PHAs who have acquired their HIV infection through mother to child transmission or contaminated blood or blood product may benefit the most from prophylactic vaccines while those PHAs who have acquired their HIV infection by sexual contact or by injection drug use may benefit less with increasing number of past sexual partners.
Incidence and mortality of cervical cancer are still high in most developing countries despite the multiple efforts to replicate the success obtained by cytology-based programs in developed countries. As a result, 85% of the half-million new cervical cancer cases in the world occur in low-resource areas and it is expected that by 2030 more than 90% of cases will be diagnosed in developing countries. In recent decades multiple researchers have developed experience with more affordable alternative screening methods that could prevent many deaths.

Visual inspection with acetic acid (VIA) has the advantage of needing very simple supplies, provides immediate results and is very well-accepted by health workers and women. Even though the sensitivity of VIA for detecting CIN2+ lesions is not optimal, the evidence shows that it is at least as good as, or even better than, cytology. VIA has also been shown to be more cost-effective than cytology when immediate treatment with cryotherapy is provided. VIA can be used as a stand-alone screening test, and in the future when new molecular tests become available in developing countries, VIA could be used as a tool for treatment selection for women with a positive screening result. Even though VIA is a very simple technique, adequate training and validation of the competency of providers is needed. The limited number of master trainers is a constraint on expansion of VIA to a national program level, but the development of training excellence centers (TEC) could overcome this barrier.

A few years ago PATH collaborated with Qiagen in the development of a rapid, simpler, and more accessible HPV-DNA test (CareHPV®). This test was validated in a rural area of China in 2007 and is currently being used in demonstration projects in India, Nicaragua, and Uganda. These demonstration projects are being developed using the public sector facilities in those countries, and the lab technicians running CareHPV are the ones currently working in that area. Preliminary results show that CareHPV is very well accepted by providers and users; its sensitivity is better than that obtained by VIA and Pap smear, even using a vaginal sample self-collected by women.

Initiating and sustaining secondary prevention cervical cancer programmes in low resource settings are fraught with complexities that relate to both human resources, infrastructure, the impact of competing health needs, lack of financial and other equipment. In addition, in many low resource settings health care is not provided free and persuading asymptomatic women to undergo screening and treatment, with limited budget, out of pocket expenses become prohibitive. In Africa, almost all cervical cancer screening activity takes place in the context of research programmes, similarly in India. Despite good data showing the efficacy and compliance with screen and treat programmes in both India and a number of African countries, widescale implementation has not occurred. This remains a resource issue and the failure of political will – until the burden of cervical cancer is appreciated at the level of resource allocation i.e government, this issue is unlikely to change. The milleneum development goals (MDGs) had a significant impact on forcing health care ministries to attend to the issue of maternal mortality, and with great effort and political will, global maternal mortality is reducing, although not in many parts of Africa. Poor countries lack sufficient health care personnel and those that exist are either in private practice or overwhelmed by the needs of people requiring curative care. In Southern and East Africa, the problem is further compounded by the massive HIV epidemic which has significantly diverted resources from preventative health interventions, such as for cervical cancer.
AGE-SPECIFIC PREVALENCE OF INFECTION WITH HUMAN PAPILLOMAVIRUS IN MALES: A GLOBAL REVIEW
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Purpose: Global data on age-specific prevalence of human papillomavirus (HPV) infection in males, especially for oncogenic HPV types 16 and 18, are essential for future efforts to prevent HPV-related diseases, including expanded access to HPV prophylactic vaccines for boys and young men.

Methods: A systematic review of peer-reviewed publications was conducted to summarize worldwide data on genital HPV-DNA prevalence in men. Studies using polymerase chain reaction or hybrid capture detection assays were included.

Results: Approximately 6,600 abstracts were identified. Sixty-four reported age-specific HPV prevalence and were included in the review. Of these, 38 were from populations at high risk of HPV infection, such as STI clinic attendees, HIV positive males, and male partners of women with HPV infection or abnormal cytology. The largest proportions of studies were from Europe (38%) and North America (25%), with smaller proportions from Central and South America (19%), Asia (11%), and Africa (5%). Across all regions, data on HPV prevalence were generally limited to men over 18 years of age. HPV prevalence was high among sexually active men in all regions but with considerable variation, from 1% to 84% among low-risk men and from 2% to 93% among high-risk men. Peak HPV prevalence spanned a wide range of ages and was generally not concentrated in the younger age groups. Age-specific prevalence curves were relatively flat or declined only slightly following peak prevalence.

Conclusions: Genital HPV infection in men varies widely, both between and within high and low risk groups and by geographic region. Compared to women, HPV prevalence in men appears to peak in slightly older ages and remains constant or decreases slightly with increasing age, suggesting persistent HPV infection or a higher rate of reinfection than that seen in women.

CAREHPV™ TEST PILOT PERFORMANCE IN RURAL NORTHERN TANZANIA
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Objective: Previously, using a prototype system, the careHPV Test (QIAGEN, Gaithersburg, USA) showed proven clinical sensitivity and specificity, compared to visual inspection with acetic acid (VIA), in a remote environment where other more sophisticated molecular HPV tests could not be performed (1). Here, for the first time, we used the final CE-marked system as a screening tool in rural Africa to determine the validity of careHPV Test results compared to those from a laboratory reference test (HC2 High-Risk HPV DNA Test®) from co-collected liquid-based cytology (LBC) specimens.

Methods: In June 2010, women aged 30-60 with no prior history of cervical cancer screening were recruited from villages surrounding Selian Lutheran Hospital in Arusha, Tanzania. After consent was obtained via local nurse-translators, the women underwent a gynecologic exam including cytology from LBC, careHPV specimen collection and testing, and VIA. careHPV specimens were tested onsite by trained local laboratory technicians. All co-collected LBC specimens were subsequently shipped to QIAGEN for reference HPV testing (HC2). HPV genotyping was performed using GP5+/6+ PCR amplification coupled to type-specific fluorescence bead detection and was used to adjudicate discordant results.

Conclusions: 324 women were enrolled in this study (mean age: 42 years; range: 30-60 years) with the majority reporting monogamous marital relationships. Most women were multiparous and 21% were postmenopausal. careHPV testing at the collection site was positive in 33/324 women (10.2%). After reference-lab HC2 results from the co-collected LBC specimens and adjudication of discordants by genotyping, positive agreement with HC2 was 90.3% (95% CI 75.1, 96.7); negative agreement was 99.6% (95% CI 98.0, 99.9); and total agreement was 98.7% (95% CI 96.8, 99.5). The kappa was 0.93 (95% CI 0.86, 1.0). Most HPV infections were single type (29/32), with HPV 16 the most common type followed by types 31 and 51. Larger, follow-up studies with clinical endpoints will help determine if the careHPV Test can be a cost-effective cervical cancer screening tool in this and other underserved African populations.

IMPLEMENTATION OF HPV VACCINATION IN LOW RESOURCE SETTINGS

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**Objectives:** HPV vaccination has the potential to reduce cancer deaths by more than 60% in developing countries, where 85% of the mortality occurs. Successful planning for HPV vaccine introduction in developing-country settings requires a comprehensive approach that addresses factors influencing HPV vaccination. In collaboration with national governments, PATH has recently completed HPV vaccine demonstration projects in India, Peru, Uganda, and Vietnam to assess different delivery strategies, including schools and health centers, to administer HPV vaccine to girls 9–14 years old.

**Methods:** We evaluated 3-dose vaccination coverage, community acceptability, feasibility of introduction within existing systems, and incremental costs for each strategy using a mixed methods quantitative and qualitative design that included a World Health Organization immunization cluster coverage survey, micro-costing, vaccination observations, interviews, and focus group discussions.

**Conclusions:** HPV vaccine coverage achieved using school-based delivery, selecting by grade, was 82% in Peru, 90% in Uganda, and 93% in Vietnam. Facility-based delivery in Vietnam achieved 98% coverage, and a combined school-facility program achieved 68%-88% in India (depending upon location). A community-based strategy, selecting girls by age, had limited success (61%) in Uganda, due to inability to accurately assess the age of eligible girls. HPV vaccine acceptability in communities was high, due to endorsement of the program by governments and community leaders, and comprehensive community sensitization and mobilization. Delivery was feasible due to good collaboration between health centers and schools for coordinated implementation, strengthening of cold chain systems, and the willingness of health workers to add HPV vaccination to their existing portfolio of services. Cost varied by country and vaccination strategy. High coverage is achievable for HPV vaccine, demonstrating the acceptability and feasibility of vaccinating young adolescent girls in developing countries.

SCREENING FOR CERVICAL CANCER USING VISUAL INSPECTION WITH ACETIC ACID OR HPV TESTING IN LOW INCOME COUNTRIES

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**Objectives:** It is estimated that, in 2008, approximately 530,000 women developed cervical cancer and that 275,000 died from the disease. 88% of all cervical cancers occur in developing countries and of all malignant tumours cervical cancer is the one that is most easily preventable by screening. For developing countries, real-time screening tests that provide an immediate result are preferable, to tests requiring transport to laboratories and multiple appointments. Moreover screening tests should be not expensive, easy to use and sufficiently accurate for identifying CIN or cervical cancer. Real time screening tests also allow for treatment to be carried out during the same clinic visit. Visual inspection with acetic acid (VIA), potentially responds to these criteria in a low resource setting. Our Cochrane review assesses the accuracy of VIA identifying CIN or cervical cancer. Real time screening tests also allow for treatment to be carried out during the same clinic visit. Visual inspection with acetic acid (VIA), potentially responds to these criteria in a low resource setting. Our Cochrane review assesses the accuracy of VIA identifying CIN or cervical cancer.

**Methods:** A literature search was performed and studies were retrieved from the electronic bibliographical databases PubMed-MEDLINE and EMBASE. The bibliographic data base CERVIX of the Unit of Cancer Epidemiology at the Scientific Institute of Public Health (Brussels, Belgium), containing more than 20,000 references was used as an additional source. Studies were included if the participants were asymptomatic women attending for cervical screening and where VIA, HC2 testing was performed. Women with a prior history of cervical treatment were excluded. For studies in which the reference test (colposcopy and biopsy) was applied to all subjects (complete studies), we extracted the numbers of true-positives, false-positives, false-negatives and true-negatives to calculate specificity and sensitivity and diagnostic odds ratio. Studies where the reference test was applied to only those with a positive screening test (incomplete studies) the number of test positives, true positives, the total number of tested subjects and the number of test positive cases with verification was extracted to compute the test-positivity rate, the ratios of positive predictive values and the detection rate ratios for CIN2+ and CIN3+. METADAS, a SAS macro f was used to estimate the pooled sensitivity and specificity for CIN2+ or CIN3+. METADAS comprises a HSROC (hierarchical summary ROC curve regression) model and a bivariate normal model incorporating the intrinsic negative correlation between the logits of the true and false positive rates.

**Conclusion:** Preliminary results from studies processed yielded a pooled sensitivity and specificity of VIA for detecting CIN2 or worse disease of 75.6% (95% CI [CI]: 69.9-80.6%) and 86.1% (CI: 83.4-88.5%), respectively. HPV-based screening with HC2 showed higher sensitivity (87.2%; CI: 79.0-93.1%) and similar specificity (88.3%; CI: 84.1-91.5%). Derived from processed studies HPV-based cervical cancer screening using HC2 appears to be more accurate than screening with VIA. Conclusions should be considered with caution given incompleteness of the meta-analysis and the risk of gold standard misclassification due to the variable quality of verification with colposcopy and biopsies.
As to its etiology. In this respect, the studies emerging since the 1980’s and suggesting a causal link between HPV and BC are of major significance among women worldwide, with ever increasing annual incidence even in developing countries, and remaining a complete enigma in vivo. Similarly, more evidence is needed before PC can be included among HPV-related malignancies. The screening program targeted rural women aged between 35 and 59 years. We compared screening frequencies with once per lifetime, 10 year intervals, 5 year intervals and 3 year intervals using the efficiency curve. Outcomes included reduction in lifetime cancer risk, life year saved (LYS), total lifetime costs and incremental cost-effectiveness ratios (ICER), presented as cost per life year saved.

Conclusion: In a rural area in China with a relatively low incidence rate, cervical cancer screening performed with conventional cytology with good quality control would still provide a greater reduction in cancer risk and be a cost-effective method regardless of the screening intervals when compared with no-screening at all.
SS 23-2

HPV AND SKIN CANCER

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Contact with viruses, solar irradiation, genetic predisposition, and increased susceptibility to immune suppression play an important role in viral tumorigenesis. Epidermodysplasia verruciformis (EV), a rare genodermatosis characterized by an abnormal susceptibility to beta human papillomaviruses (HPV) and skin cancer associated with HPV5, constitutes a good model for HPV-induced skin carcinogenesis. Genetically determined defects in immunosurveillance of the infection with beta-HPV are observed in the disease. The role of HPV in nonmelanoma skin cancer (NMSC) of immune competent hosts is more difficult to prove. This is due to the widespread infection of normal skin with HPV and the low copy number of viral genomes in tumors while UV irradiation, immunosuppression, light color skin and genetic background are considered as major risk factors for NMSC. In addition, the weak transforming activity of beta-HPV contrasts to high-risk genital HPV. However, a carcinogenic role of HPV in early steps of tumor development has been hypothesized. This review will focus on epidemiology and mechanisms of NMSC associated with HPV.

SS 23-3

HUMAN PAPILLOMAVIRUS (HPV) INVOLVEMENT IN BRONCHIAL CARCINOGENESIS

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Bronchial squamous cell carcinoma (BSCC) is a major disease burden worldwide, showing alarmingly increasing trends particularly among women in many societies. Epidemiological and experimental data suggest that cigarette smoke, radiation, and asbestos exposure are the prime aetiological agents associated with the development of this malignancy. Since the original reports of this author (dating back to 1979), providing the first evidence to suggest HPV involvement in etiology of a subset of bronchial carcinomas (Respiration 1979;38:299–304; Lung 1980;158:131-142; Respiration 1980;40:150–60), however, the studies on HPV detection in bronchial cancer have increased rapidly. Based on data summarised recently (J. Clin. Pathol. 2002;55:885-891), the evidence for the involvement of HPV in bronchial carcinogenesis has accumulated through several distinct lines of research: 1) HPV DNA has been detected in around 50% of benign bronchial squamous cell papillomas (SCP); 2) the detection of morphological changes suggesting HPV in bronchial cancer and its precursor lesions on light microscopy; 3) the expression of HPV structural proteins by immunohistochemistry; 4) the detection of HPV DNA and RNA by different hybridisation assays and PCR; and 5) in vitro studies, e.g. transformation of bronchial epithelial cells by oncogenic HPV types. Not unlike in other non-genital lesions, HPV detection rates in bronchial carcinomas are highly variable in the different studies published from several countries, ranging from 0% to 100%. The literature contains 2,468 bronchial carcinomas subjected to HPV detection and HPV DNA has been reported in 536 (21.7%) of these cases. More recently, also the expression of HPV E6 and E7 oncogenes has been confirmed in BSCC. The incomplete experimental data accumulated so far suggest that similar mechanisms as those detected in HPV associated cervical carcinogenesis might also be involved in bronchial carcinogenesis. An HPV18-immortalised bronchial cell line (BEP2D) has recently been studied as a model of multistep bronchial carcinogenesis induced by radiation and asbestos fibres. There is reason to suspect that bronchial carcinogenesis is a multistep process, which is contributed to by known pathogenetic factors (cigarette smoke, radiation, asbestos exposure). The addition of HPV to the list of potential carcinogens of the bronchus deserves far more serious attention than it has obtained so far.
It has been well established that HPV infection is the single most important aetiological factor of cervical and anal cancers. Apart from these ano-genital lesions, this virus has been implicated in the pathogenesis of squamous cell lesions of the skin, oral cavity and upper aero-digestive tracts.

HPV involvement in oesophageal carcinogenesis has been demonstrated by morphologic studies showing koliocytic changes in benign and malignant squamous lesions, by immunohistochemical staining for HPV antigens, by DNA hybridization disclosing HPV DNA sequences, by sero-epidemiological studies showing an increased risk of oesophageal cancer in relation to oncogenic HPV exposure and by in vitro studies showing transformation of oesophageal epithelial cells by oncogenic HPV types. Of several thousands of oesophageal squamous carcinomas analysed, HPV detection rates are 27% by in situ hybridization and 33% by PCR.

An intriguing observation is that HPV detection rates in oesophageal carcinomas appear highly variable in different geographical areas of the world, being significantly higher in high-risk areas than in low-risk regions. Interestingly, a similar disease pattern has been found from animal studies on cattle, particularly in the high-incidence area of Scottish Highlands, where bovine papillomavirus (BPV) type 4 infection has been linked to the development of oesophageal papillomatosis and carcinomas. Apart from BPV infection, the ingestion of bracken fern was found to be a crucial co-factor in this malignant transformation.

Globally, oesophageal squamous cell carcinoma shows a marked geographic variation, with up to 500-fold difference in incidence between low- and high-risk areas. Such an uneven distribution suggests a dominant role of external environmental factors in its aetiology. It is generally accepted that oesophageal carcinogenesis is a multistep process and the development of an invasive oesophageal carcinoma represents an ultimate result from synergistic actions between multiple aetiological factors. Infection by the oncogenic HPV types appears to play a causal role in the development of oesophageal carcinoma.

Recurrent respiratory papillomas of the larynx are caused by HPV-6 and -11. Juvenile laryngeal papillomas, though rare, can develop in neonates and infants up to age 7 in children born to mothers with active cervico-vaginal HPV infections, and with evidence that some infections initiate in utero. Adult-onset disease most likely occurs as a result of sexual transmission. The reasons for low infection rates and viral expression in upper aerodigestive tract tissues are not known. Nonetheless, the consequences of active infections are severe: surgeries to remove papillomas that compromise the voice and obstruct the airway often lead to regrowth of lesions from subclinical HPV infections in the wound margins. Young patients can require surgeries as frequently as monthly, while recurrence in adults is generally protracted. Respiratory papillomas may regress and become subclinical after years of activity, but they can reactivate as a result of irradiation of or injury to the vocal cords or during pregnancy. HPV-11 infections are more common in infants and young children than in adolescents and adults, more aggressive than those caused by HPV-6, and more likely to migrate or activate further down the airway to cause tracheal, bronchial and rarely pulmonary lesions, where malignant transformation can occur. Eosophageal involvement is also associated with aggressive laryngeal papillomas. There is inadequate public and professional awareness of HPV lesions of the airway and an urgent need for hospital (especially ER) staff and pediatricians to learn about HPV, as they are often the first to see an RRP patient. OB-GYNs should become more familiar with RRP, especially regarding ways to minimize transmission of the papillomavirus during birthing.

As adjuvant therapies to ablative surgeries, patients have been treated with interferon-alpha, retinoids to prevent squamous differentiation, intralesional injections of the acyclic nucleoside phosphonate analog cidofovir to inhibit RNA and DNA polymerases, the botanical indole-3-carbinol and its metabolite diindolelylmethane, artemisinin, artsunate and analogues to induce reactive free-radicals and oxidative stress in the affected tissues, the COX II inhibitor celecoxib to block production of prostaglandin E2, and bevacizumab (Avastin) to inhibit capillary regrowth after angiolytic KTB laser treatment. Intra-lesion injections of the mumps virus or MMR vaccine have led to remarkable inhibition of HPV activities. However, no single treatment is successful for most patients, and finding beneficial adjuvants proceeds by trial and error. The quadravalent HPV vaccine should diminish H&N and respiratory papillomas and carcinomas, once a high fraction of people become vaccinated. Worldwide management of RRP and other H&N infections with HPV demand research on and development of effective therapeutic vaccines and small molecule inhibitors of viral persistence, early gene expression and DNA replication.
INVESTIGATING THE ROLE OF HPV IN LUNG CANCER: A POOLED ANALYSIS

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Objectives: Lung cancer is the leading cause of cancer mortality worldwide and cigarette smoking is the primary risk factor; there are gender differences in the relationship between smoking and lung cancer, as well as in response to therapy. Human Papillomavirus (HPV) is the etiologic risk factor for cervical cancer; some studies have suggested that HPV may be also associated with a subset of lung tumors, although an etiologic link has not been firmly established. We performed a pooled analysis of cross-sectional studies to evaluate the prevalence of HPV in lung tumors and to determine whether there is a difference in prevalence across levels of smoking status and gender.

Methods and Results: We report preliminary results from a pool of five datasets from four published and one unpublished study (n = 353) out of 12 potential eligible studies involving 1,499 lung cancers. The HPV status was determined using polymerase chain reaction (PCR) protocols to detect HPV DNA (4 studies) or RT-PCR to detect HPV RNA transcripts (one study). Seventy-eight percent of all cases were enrolled in studies conducted in Europe (n = 277) and 22% of the cases were enrolled in the single study conducted in the United States (n = 76). The mean age of the study population was 56 years old and the majority of cases were male (74%, n = 260). The histologic types were primarily non-small cell carcinoma with a predominance of squamous cell carcinoma (n = 199, 56%) and adenocarcinoma (116, 33%). The overall HPV16/18 prevalence was 4%. After adjusting for study, smoking and age, the prevalence of HPV16/18 positive lung tumors was slightly higher in males compared to females (0.7% (0.0-11.6) vs. 0.3% (0.0-8.6)). Ever smokers were slightly more likely to have lung tumors positive for HPV16/18 compared to never smokers (2.6%, (0.7-9.9) vs. 1.9%, (0.2-17.1)); the prevalence of HPV16/18 positive lung tumors also increased with age (35-54 yrs: 0.1% (0.0-4.3); 55-64 yrs: 0.3% (0.0-7.8); 65-73 yrs: 1.7% (0.0-23.5); 74-86 yrs: 1.6% (0.0-23.1)). Adenocarcinoma cases had a higher proportion of HPV-positive lung tumors compared to squamous cell carcinoma cases (1.2% (0.0-16.3) vs. 0.7% (0.0-9.7)).

Conclusions: Our findings confirm the presence of HPV infection in lung tumors, and suggest possible differences in HPV prevalence with gender. Further analyses of the larger, complete dataset may allow the study of the interaction between HPV and smoking in lung cancer development.

DETECTION OF HUMAN PAPILLOMAVIRUS TYPE 16 DNA BUT ABSENCE OF EXPRESSION OF E5, E6 AND E7 VIRAL mRNA IN COLORECTAL CANCERS

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Background: Over the last few years a possible correlation between HPV and colorectal cancer has been suggested. HPV infection has been reported by several studies with a significant difference between tumors and controls. The most prevalent genotype reported was HPV 16.

Aims of our study were to investigate: the presence HPV type 16 DNA in colorectal carcinomas and resection margins, and the mRNA expression of E5, E6 and E7 in positive tissues, in order to correlate a possible HPV infection with clinical and prognostic factors.

Materials and Methods: After DNA extraction from 29 FFPE tissues of 28 patients (M/F 1.5, mean age 63.3 yrs, range 35-80) undergone to lower anterior/miles resections or subtotal colectomy for colorectal cancer, Real-Time PCR for HPV 16 DNA (primers for E6/E7 region) was performed. Surgical resection margins were also investigated in HPV positive cancers. Moreover, in positive cases, RNA from frozen tissue was extracted and analysed with reverse Real-time PCR for HPV 16 E5, E6 and E7.

Results: HPV 16 DNA was detected in 3 tumors (10.3%), 2 of Stage I (G2 and G3), 1 of Stage II (G3). Proximal margins were found positive in one patient, while distal margins were positive in two patients. Although human DNA was positively validated by the housekeeping, our analysis on the expression of E5, E6 and E7 (performed by RT PCR on RNA from frozen tissues of the positive cases) failed in detecting the HPV mRNAs within tumors and adjacent tissues.

Discussion: HPV type 16 infection rate in colorectal cancers was 10.34%, lower if compared with past reports. This might be referred to differences in primers set or in primer efficiency due to FFPE tissues. We didn’t find a significant difference in distribution of the virus through the colon segments, however, due to the small number of patients, we could not stratified groups.

Conclusion: This is the first Italian study investigating the association of HPV and colorectal cancer, and the first study aimed to correlate viral HPV DNA and mRNA expression through Real-time PCR, but further studies will be required to better investigate the role of HPV in colorectal cancers, the integration of the virus and the expression of viral proteins in positive cancers.
INVESTIGATION OF GENITAL HPV INFECTION IN CYPROT MEN AND IN MALE UROGENITAL CANCERS

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Objective: Human Papillomavirus (HPV) causes anogenital and oropharyngeal cancers. Our previous studies in the general population of Cypriot women determined types and frequency of HPV infection as well as risk factors for HPV infection, condylomata and cervical neoplasia (Neophytou et al., 2006). In the present study we have investigated HPV infection in Cypriot men. In addition we have analyzed HPV DNA presence in urinary bladder and prostate tumours in order to investigate HPV infection in the etiology of these malignancies.

Methods: We used an MY09/11-based PCR/RFLP system, which has obtained excellent marks in the European HPV DNA test External Quality Assurance Scheme (EHEQAS). We tested urine and/or genital swabs from 389 men who had genital condylomata or whose sexual partner had a recent HPV infection. Furthermore biopsies of 42 urinary bladder tumours and 113 prostate tumours were analyzed.

Results: Out of 389 men 93 (24%) were HPV positive. The level of infection was 11% in men without and 52% in men with a history of condylomata. More than twenty different HPV types were detected. The frequency of infection by HPV 11 in Greek-Cypriot men was 14%, while it is only 2% in Greek-Cypriot women. Out of 42 bladder tumours tested (including 39 invasive cancers) none were HPV positive, while out of 113 prostate tumours (13 invasive cancers, 2 prostate intraepithelial neoplasia, 98 benign prostate hyperplasia) 4 were HPV-positive (1 invasive cancer, 3 benign prostate hyperplasia).

Conclusion: The most important risk-factor for genital HPV infection in men is a history of condylomata, whereas other factors examined (multiple sexual partners, smoking, age at first coitus, other genital infections, family history of cancer etc.) did not reach statistical significance. HPV 11 has a significantly increased frequency in Greek-Cypriot men than in women. HPV does not play a role in the pathogenesis of urinary bladder and prostate cancers.

EVALUATION OF SAMPLING METHODS FOR DETECTION OF ANOGENITAL HPV IN MEN WHO HAVE SEX WITH MEN

Stephen Goldstone

Background: Although there exists a strong relationship between HPV types 16 and 18 and anal cancer and HPV types 6 and 11 and genital warts, the specific anogenital regions of the male that serve as the primary reservoirs for these HPV types remained to be determined as well as the best way to detect HPV at male external genital sites. The primary goal of this study was to evaluate a sampling technique for men which was both effective in detecting HPV infection and acceptable to subjects participating in clinical trials.

Methods: The study enrolled 40 men who have sex with men (MSM) at high risk for HPV undergoing anogenital screening and/or treatment for HPV infection. There was only 1 visit in this study. This visit included urine, semen and serum collection for detection of HPV antibodies. Anogenital specimen collection from the penis, scrotum, perianal and anal region was performed with a wetted DACRON™ swab after gentle rubbing with file. Examination for external genital lesions, anal cytology and high-resolution anoscopy (if indicated) was also conducted.

Results: In general, the sampling technique evaluated in this study detected HPV DNA in 90% of study subjects. Sampling in the perianal/anal regions was found to be most sensitive for detection of HPV DNA. In addition, the file and swab technique was well tolerated by subjects.

Conclusions: The swab sampling technique is adequately sensitive and is sufficient for use in clinical trials. Collecting samples from multiple anogenital areas increases sensitivity of HPV DNA detection in men.
HUMAN PAPILLOMAVIRUS TESTING IN PRIMARY CERVICAL SCREENING AND THE CUT-OFF FOR HYBRID CAPTURE 2 TESTS: A SYSTEMATIC REVIEW

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Objectives: Randomized controlled trials (RCT) on primary cervical screening with Hybrid Capture 2 (HC2) Human Papillomavirus (HPV) DNA testing suggested that HC2 using the cut-off ≥1 rlu/co is highly sensitive but leads to many positive tests without underlying high-grade cervical intraepithelial neoplasia (CIN). Using these data, the aim was to determine the trade-offs between a decreased sensitivity for ≥CIN3 and ≥CIN2, and a decreased frequency of positive HC2 tests not associated with these CIN for a range of increased HC2 cut-offs compared to ≥1 rlu/co.

Methods: We identified potentially relevant articles in PubMed, using terms “HPV” or “Human Papillomavirus” and the names of principal RCT investigators. In the analysis, we included articles published until August 2010 which stratified the baseline-round number of positive HC2 tests and CIN by HC2 cut-off level.

Conclusions: HC2 data stratified by cut-off were reported from 4 RCTs, and 25 observation points within the range of cut-off points between ≥1 rlu/co and ≥10 rlu/co were available for analysis. The relative ≥CIN3 and ≥CIN2 detection rates at cut-offs ≥2, ≥4-5, and ≥10 rlu/co compared to cut-off ≥1 rlu/co ranged between 0.97 and 1.00, between 0.92 and 1.00, and between 0.91 and 0.96, respectively. Up to 24%, 39%, and 53%, respectively, of positive HC2 tests not associated with these CIN could be avoided at these cut-offs. There were only 2 outliers to this general pattern. The decrease in sensitivity for high-grade CIN using a HC2 cut-off between ≥2 rlu/co and ≥10 rlu/co appears acceptable given the international HPV DNA testing recommendations in cervical screening, which require ≥90% detection compared to HC2 at ≥1rlu/co. At the same time, the number of positive tests without an underlying CIN lesion could be reduced considerably at these higher cut-offs. The HC2 cut-off could be increased in primary screening.

QIASICMPHONY AXPH SAMPLE PREPARATION FOR AUTOMATED CONVERSION OF LIQUID BASED CYTOLOGY SPECIMENS FOR USE IN THE DIGENE HC2 HIGH-RISK HPV DNA TEST

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Cervical specimens collected in PreservCyt or SurePath media are routinely used for cervical cancer screening. In Europe, both liquid based cytology (LBC) media are validated for use with the digene HC2 High-Risk HPV DNA Test* (HC2). The current HC2 specimen conversion protocols for PreservCyt and SurePath require manual sample processing. QIAGEN has developed and validated a Clinical Sample Concentrator and CE-IVD certified LBC extraction procedure based on pH-driven anion exchange sample preparation chemistry (AXpH) for use on the QIAsymphony SP. This presentation summarizes activities to develop the QIAsymphony AXpH procedure and to demonstrate its usability as fully automated front end for LBC-based HC2 testing.

Specimens collected in PreservCyt or SurePath liquid based cytology medium require different sample conversion procedures for use in HC2. Manual and automated workflow analysis for each specimen type will be presented. Performance characteristics including QIAsymphony DSP AXpH DNA Kit* stability, eluate stability, sample stability, repeatability and the influence of potentially interfering substances on HC2 testing will be shown for both specimen types.

To compare manual with automated sample conversion methods, residual, de-identified, clinical PreservCyt and post-gradient SurePath specimens, retained after cytology screening, were processed. DNA was isolated on the QIAsymphony using the respective AXpH protocol. The established manual sample conversion method was used as a reference. The QIAsymphony eluates and the corresponding manually converted pellets were tested with HC2. Positive, negative and total agreement with the manual reference method will be presented.

The shown performance characteristics of the QIAsymphony DSP AXpH DNA Kit indicate that automation of LBC specimen conversion for use in HC2 testing is feasible.

*CE-marked for In vitro diagnostics use in Europe. The combination of QIAsymphony AXpH and digene HC2 High-Risk HPV DNA Test presented here is currently for research use only. Not for use in diagnostic procedures.
LIMITATIONS OF WIDELY USED HIGH RISK HPV DNA TESTING IN PATIENTS WITH INVASIVE CERVICAL CANCER

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**Objectives:** To understand the basis for false negative HPV test results reported in a young woman diagnosed with cervical cancer.

**Methods:** Two cervicovaginal samples were collected in SurePath™ (Becton Dickinson, Franklin Lakes, NJ) vials, 2.5 years and 1 month prior to diagnosis of cervical cancer by histopathology from colposcopic biopsy. The first cytology specimen was ASC-H; the second HSIL. High risk HPV testing by Hybrid Capture 2 (hc2) (Digene, Gaithersburg, MD) and PCR was performed. Published literature on validation of laboratory developed testing (LDT) for HPV DNA from the non-FDA-approved SurePath vial, test validation data from national laboratory websites, phone surveys and data from SeraCare Life Sciences (Gaithersburg, MD) the HPV Proficiency testing vendor of the College of American Pathologists were reviewed.

**Results:** Negative hc2 HPV results were reported for both residual SurePath vial specimens. HPV DNA was identified by PCR. Limited published data on hc2 HPV testing from the SurePath vial indicates false negative HPV results in some cervical cancer patients. HPV proficiency testing vendor documented HPV 16 DNA degradation in SurePath vials. Others have reported that HPV DNA fixation in the SurePath vial may interfere with HPV DNA testing. Validation studies required under federal regulations for LDT are often inadequate, commonly document limited analytic test performance, and usually lack clinical validation data. No laboratory survey data has documented stability of HPV testing from the SurePath vial over several weeks’ time in low or high viral load patients with significant histopathologic lesions. These data contrast with reports using FDA-cleared HPV test methodology.

**Conclusions:** Non FDA-cleared HPV DNA testing is widespread in the USA, most often utilizing hc2 HPV testing from the SurePath vial. Clinical validation data supporting reliable test performance in patients with cervical cancer is unavailable, whereas false negative reports predominate. Absence of user validation data documenting test stability over time is concerning, and raise the possibility that components in the SurePath process may interfere with HPV DNA testing by time-dependent DNA degradation and/or DNA fixation. Research is needed on this unrecognized patient safety issue.


**SS 24-5**

PERFORMANCE OF BD VIPER™ HPV ASSAY USING FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) SAMPLES

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**Objectives:** The new BD VIPER™ HPV assay has an automated extraction chemistry which is designed to directly process liquid based cytology specimens without the need for cell recovery or the use of Proteinase K prior to amplification. This study was designed to assess the ability of the assay to detect HPV DNA directly in formalin-fixed paraffin-embedded (FFPE) tissue and to assess its feasibility for detecting other target analytes in this specimen type.

**Methods:** FFPE tissue is a challenging sample type due to the cross-linking effects of formalin and the fact that the extracted nucleic acid is often of low molecular weight. Standard sample preparation methods often require the use of xylene washes to remove paraffin and Proteinase K to remove protein-DNA cross-links. We have developed a rapid, automated, single-step method that obviates the need for pre-treatment or protease steps which utilizes direct chemical lysis to extract the DNA. The ability of this method to detect both target HPV DNA and human cellular DNA in fixed tissue sections was assessed using 3 x 5 \(\mu\)m sections.

**Results and Conclusions:** Previously characterized FFPE sections from cervical cancer biopsies were diluted with distilled water and processed in BD HPV diluent using our standard assay procedure. The automated BD Viper HPV method was found to have excellent correlation with other labor-intensive manual methods and consistently detected both human beta globin (internal control) and viral HPV targets. Beta globin Ct scores were in line with those observed in cytology specimens and the assay was capable of detecting multiple HPV genotypes when present in the specimen.
EVALUATION OF THE REALTIME HIGH RISK HPV ASSAY WITH SIMULTANEOUS TYPING OF HPV16 AND HPV18 IN WOMEN WITH ATYPICAL PAP SMEAR

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Objectives: This study investigates the performance of the automated Abbott RealTime High Risk HPV assay (RealTime-HPV) by comparison to Qiagen/digene hybrid capture 2 (hc2).

Methods: A QCMD Panel and 545 patient samples (ThinPrep) from two German colposcopy clinics were evaluated. Patient samples were tested with hc2, targeting 13 high-risk (HR) HPV types without individual typing and RealTime-HPV, detecting 14 HR-HPV types and simultaneously differentiating between HPV16, HPV18 and a pool of 12 other HR-HPV types in a single test on the m2000 System. Discordant samples were retested with both assays and repeat discrepant cases were resolved by genotyping (Roche Linear Array). Analytical performance parameters were calculated based on data from 545 samples. HR-HPV detection rates were determined based on histological findings from 319 cases (90 non-pathological, 72 CIN1, 75 CIN2, 75 CIN3 and 7 invasive cancers).

Results: RealTime-HPV identified all QCMD panel members containing targeted HPV genotypes at concentration levels specified by the supplier. No cross-reactivity was observed with a non-targeted type (HPV 67). Good overall-agreement (92.8%; k: 0.86) was found between RealTime-HPV and hc2 on 545 patient specimens. Relative analytical sensitivity, specificity and accuracy observed with RealTime-HPV (98.8%, 100%, 99.5%) were significantly higher than with hc2 (94.4%, 91.7%, 93.0%). HR-HPV detection rates in <CIN2, CIN2+ and CIN3+ were 38.3%, 92.4% and 97.6% with RealTime-HPV and 41.4%, 91.7% and 92.7% with hc2. HPV16/18 detection rates were highly correlated with increasing severity of dysplasia: <CIN2 13.6%, CIN2 + 59.9%, CIN3 + 72.0%. Clinical sensitivity of RealTime-HPV for CIN2+ (92.4%) and CIN3+ (97.6%) was comparable to hc2 (CIN2+ 91.7%, CIN3+ 92.7%).

Conclusions: RealTime-HPV showed clinical performance comparable to hc2 and analytical performance superior to hc2 (no cross-reactivity to low-risk HPV). High level automation with a turn-around-time of <6 hours (96 tests) and the capability of distinguishing between HPV16, HPV18 and other HR-HPV types support the use of RealTime-HPV for clinical evaluation of HPV infections.

USE OF HPV ONCOTECT® FOR PRE-SQUAMOUS CELL CARCINOMA AND PRE-ADENOCARCINOMA DETECTION AND DIFFERENTIATION

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Background: Cervical cancer encompasses both squamous cell carcinoma and adenocarcinoma. Though the majority of cervical cancers are squamous cells carcinoma, adenocarcinoma rates are on the rise in some demographics. Though HPV DNA testing has improved the performance of cervical cancer screening, HPV DNA is positive in only 35% of atypical glandular cells (AGC) cases. Further a HPV positive test in women with AGC is more likely to reflect CIN2/3 rather than AGC associated disease (AGC-AD).

Methods/Results: We are performing a prospective analysis of 82 women with AGC. To determine the performance of HPV Oncotect for the detection of pre-adenocarcinoma/adenocarcinoma of the cervix, we analyzed 82 samples of AGC with HPV Oncotect using novel software modifications that separately determine E6, E7 mRNA overexpression in ectocervical cells and endocervical cells in the same sample. We also performed Hybrid Capture 2 (HC2) on all samples and compared the results to cytology diagnosis and biopsy/endocervical curatage. HPV Oncotect EndoGate was positive in 38 (46%) of AGC cases including all 4 (100%) adenocarcinoma cases and HC2 was positive in 44 (54%) AGC cases but 3 (7%) of adenocarcinomas. The increased positivity of HC2 is due to the detection of CIN 1-3 not detected by HPV Oncotect EndoGate though detected by HPV Oncotect in the squamous cell gating.

Conclusions: HPV Oncotect can detect AGC cases with underlying high grade endocervical diseases and can discriminate between AGC cases with endocervical disease from AGC cases with squamous cell disease.
**SS 24-8**

**CLINICAL EVALUATION OF THE STANDARDISED GP5+/6+ EIA KIT**

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**Objectives:** High-risk (hr) HPV testing is a valuable tool for population-based screening for cervical cancer. Two of the most studied and, clinically validated assays are the commercially available Hybrid Capture 2 (HC2; QIAGEN) and the in-house GP5+/6+ EIA assay (EIA). Both have demonstrated equal clinical sensitivity, but the EIA is clinically more specific. Without modifications, the original in-house EIA test is now available as a standardised CE-marked kit, produced under ISO certified conditions (EIA kit HPV GP HR, Diassay BV). The aim of this study was to perform a clinical evaluation of this kit.

**Methods:** Specimens were collected from women (n=184), who were referred for colposcopic evaluation due to an abnormal Pap smear or HPV infection detected at basic healthcare centres. First, a cervical smear was obtained and stored in ThinPrep medium. Aliquots were used for cytology, HC2 analysis, and GP5+/6+ EIA testing. The same GP5+/6+ amplimers as used for the EIA were genotyped by the digene HPV Genotyping LQ Test (LQ Test; QIAGEN). Histopathologic diagnosis was carried out on biopsies collected during subsequent colposcopy.

**Conclusions:** Overall, hrHPV positivity by HC2 (142/184; 77%) was significantly higher as compared to the EIA (118/184; 64%) (p<0.001). In the ≤LSIL group (n=122), the detection rate by HC2 (66%) was significantly higher than that of the EIA (48%) (p<0.001). In the ≥HSIL group (n=62), detection rates of HC2 (100%) and EIA (97%) were similar (p=0.5). Both hrHPV detection assays were also compared according to the histological diagnosis of the biopsy. The <CIN2 group (n=136) included 20 women without apparent lesions during colposcopy. In this group the hrHPV detection rate by HC2 (69%) was significantly higher than that of the EIA (52%) (p<0.001). In the ≥CIN2 group (n=48), positivity rates by HC2 (100%) and EIA (98%) did not differ significantly (p=1). Using the same amplimers, the LQ test could identify the HPV genotype in 115 of the 118 (97.5%) EIA-positive samples. Thirteen of the 14 hrHPV types included in the EIA probe cocktail were identified in this population. In summary, results from this study are consistent with previous findings showing the equal clinical sensitivity and higher clinical specificity of the EIA kit compared to HC2.

**SS 24-9**

**HPV TESTING WITH CYTOLOGY TRIAGE IN CERVICAL SCREENING: INFLUENCE OF REVEALED VERSUS CONCEALED HPV STATUS**

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**Objectives:** Evidence is mounting for primary cervical screening with Human Papillomavirus (HPV) DNA testing followed by triage with Papanicolaou (Pap) cytology. Since Pap interpretation is subjective in nature, revealing HPV-positivity could influence interpretation of cellular abnormalities. We simulated this triage algorithm to assess the diagnostic accuracy of Pap cytology when cytotechnicians were aware of the patient’s cervical HPV status.

**Methods:** We used existing specimens and clinical data from the Newfoundland component of the Canadian Cervical Cancer Screening Trial (CCCaST). Slides were re-read with knowledge of HPV status for all HPV-positive women (n=278) and a control sample of HPV-negative women (n=278). Cervical disease was diagnosed via a uniform colposcopy and biopsy protocol. Un-weighted Kappa tests measured agreement between original readings and re-reads. We used verification bias corrections to derive test estimates. Diagnostic accuracy of Pap cytology was compared and modeled.

**Results:** Overall agreement between original Pap readings and re-reads was 81.2% (Kappa=0.45) in HPV-positive women. Proportions classified Pap-positive were higher when HPV-positivity was revealed versus concealed for cytology cut-offs of ASC-US (24.9% vs. 15.4%) and LSIL (12.3% vs. 8.5%). Re-reads resulted in more false positives which reduced specificity from 85.2% [95%CI=80.8,89.5] to 75.3% [95%CI=69.9,80.7] for ASC-US, and from 92.6% [95%CI=89.3,95.8] to 88.7% [95%CI=84.7,92.6] for LSIL. We further examined the influence of factors related to the patient and laboratory.

**Conclusions:** We observed a decline in Pap cytology specificity when the patient’s cervical HPV-positivity was revealed to cytotechnicians. This was possibly a result of the heightened awareness of potential abnormalities.
Objective: Testing for high-risk human papillomavirus (HPV) is recommended by the American Society for Colposcopy and Cervical Pathology in two principal settings: “reflex” testing of samples with a cytologic interpretation of atypical squamous cells of undetermined significance (ASC-US), and routine screening (“co-testing”) of samples from women aged 30 years or older. Cervista HPV HR (Hologic, Inc.), a high-risk HPV DNA assay, was approved for clinical use by the US Food and Drug Administration in March 2009. Given its recent approval, limited data are available describing the clinical performance of the assay using specimens preserved in different liquid-based collection media. The objective of this study was to review and compare Cervista HPV HR test results from specimens collected in either ThinPrep medium (PreservCyt; Hologic, Inc.) or SurePath medium (Becton, Dickinson and Company).

Methods: This was a retrospective study of 56,501 samples analyzed by both cytologic examination and the Cervista HPV HR assay. Of the specimens analyzed, 37,574 were preserved in ThinPrep medium and 18,927 were preserved in SurePath medium. The Cervista HPV HR assay was performed according to the manufacturer’s instructions.

Results and Conclusions: Of the specimens analyzed, 2.6% (983/37,574) of specimens in ThinPrep medium and 2.4% (453/18,927) of specimens in SurePath medium did not yield a reportable result, due either to insufficient specimen volume or to a low signal from the assay’s internal control. A reportable Cervista HPV HR result was obtained from 97.4% (36,591/37,574) of ThinPrep and 97.6% (18,474/18,927) of SurePath specimens. Of specimens with ASC-US cytology, 47.6% (3285/6906) of specimens in ThinPrep medium, and 51.4% (1155/2245) of specimens in SurePath medium yielded a positive HPV HR result. Of specimens from women aged 30 years or older with negative cytology, 6.4% (1623/25,486) of ThinPrep specimens and 4.7% (666/14,133) of SurePath specimens yielded a positive HPV HR result. Although this study is an indirect comparison, our data suggest that performance of the Cervista HPV HR assay is similar for specimens collected in ThinPrep and SurePath media, both in terms of the proportion of specimens yielding a reportable result and in the rate of high-risk HPV positivity.

Introduction: Laboratories offering diagnostic services should have robust programmes of quality assurance to ensure that reported results are correct. Components include internal quality control, internal and external quality assessment (EQA) / proficiency testing to challenge the effectiveness of programmes. Participation in independent EQA schemes should be a mandatory part of local QAPs. In the UK, EQA of cytological diagnosis of cervical cancer is well established and imminent roll-out of HPV testing for triage and test of cure necessitates an equally robust HPV EQA scheme.

Methods and Results: Clinical EQA: use of clinical or simulated clinical samples mirrors the clinical and diagnostic environment. We assessed the suitability of residual cervical LBC samples as EQA specimens through the NHS Cervical Screening Committee HPV/LBC Pilot study and in conjunction with UK NEQAS. Residual samples were tested twice in different assay runs, pooled/diluted to produce sufficient volume and re-tested using a range of HPV assays to ensure reproducible results by checking pre-distribution in two HPV reference centres. Three pilot distributions over 2008-09 showed good inter-laboratory reproducibility across >40 laboratories in different countries using combinations of >8 different assays. We also assessed the storage and transport conditions needed to ensure robustness of clinical specimens for EQA.

Analytical EQA: cell lines and plasma-derived material containing known copy numbers of specific HPV types allow quantitative assessment of assays and are particularly useful for surveillance settings. However, analytical sensitivity of HPV tests does not match with clinical sensitivity for detection of high grade disease.

Conclusions
• Increasing use of highly sensitive HPV assays requires careful laboratory validation, including availability of robust HPV EQA schemes
• Well characterised internationally assessed quality control panels are essential to compare the various diagnostic methods
• EQA schemes using plasmid derived material are important for surveillance, while clinically based material is of particular value in cervical screening due to great variety in HPV types, multiplicity of infection and variation in viral load
• The UKNEQAS HPV scheme provides a homogenous and characterised specimen relevant to the clinical needs of participants

VALIDATION AND QUALITY ASSESSMENT OF HPV TESTING ASSAYS

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For primary screening purposes HPV testing is only useful when a positive HPV test result is informative about the presence of cervical (pre)cancerous lesions. We currently know that various HPV detection methods differ in their clinical performance of detecting HPV-related premalignant disease. The HPV tests that in large clinical trials have shown to perform better in the detection of CIN2+/CIN3+ than cytology and thus proved to be suited as primary screening test are the HC2 and GP5+/6+-PCR EIA assays. Consequently, these tests are now considered as alternative clinically validated screening tools for cytology in cervical screening. Recently, criteria of HPV test requirements have been formulated in guidelines and were translated into a validation procedure by an international consortium. This validation procedure enables validation of candidate HPV tests for screening purposes without the need of performing costly and time-consuming prospective screening trials. The guidelines indicate that in order to be considered as clinically validated a candidate HPV test should prove not to be inferior to HC2 (or GP5+/6+-PCR EIA) in terms of clinical sensitivity and specificity for CIN2+. This can be assessed in a clinical equivalence study on representative sets of women in a population-based screening cohort, tested by HC2 (or GP5+/6+-PCR EIA) and the candidate HPV test (Meijer et al., Int J Cancer 2009). Moreover, candidate tests should display a sufficient intra-laboratory reproducibility and inter-laboratory agreement. Here, guidelines for hrHPV test requirements and clinical validation of candidate hrHPV tests will be further discussed, as well as laboratory guidelines for HPV testing.

QUALITY ASSESSMENT OF HPV TESTING

Ifner T

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Commercially available HPV tests differ largely in their analytical sensitivity and quality. They can be categorized in three classes: the first detecting an infection with high-risk (HR) HPV, the second detecting not only the infection with HR-HPV but also differing between HPV 16, 18 and other high-risk types and third tests able to genotype. Some of the tests deliver the positive result only if a clinical defined cut-off value is exceeded. This enhances the specificity without affecting the sensitivity. Such a cut-off value allows only the clinical relevant finding to be pursued. Most of the tests are compared in their performance to the Digene Hybrid Capture 2 (Qiagen) which was the first test being FDA approved in the US for screening. In Europe such an authority is lacking. There are too many HPV tests conducted as inhouse PCRs without sufficient quality assurance. The regular participation of accredited laboratories at intercomparison programmes represents in fact no solid standard quality assurance in every country as for example in Germany.

Even with one HPV-detection method used by 7 different laboratories there were orders of magnitude of variation in the sensitivity to detect HPV 16 (Quint et al 2005).

To effectively reduce the incidence and mortality from cervical cancer HPV testing should become part of the national screening programmes. However for this to be achieved, HPV-tests recommend for screening need to have a proven clinical sensitivity and specificity with sufficiently large trials published and laboratories performing the test should undergo a QA-testing with HPV reference panels including more than just two HR types.
SS 25-4

GENOTYPING PREVALENCE BY COUNTRY

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To evaluate HPV prevalence and type specific distribution of HPV infection in the general population in Italy, 4 different HPV prevalence surveys were financed by Minister of health:
- NTCC study: HPV prevalence and type distribution in over 50,000 women aged 25-60 participating in the Italian screening program for cervical cancer
- HPV prevalence and type distribution in center and south Italy in 4,000 women aged 25-64 years
- Typing 1,000 CIN2+ lesion on tissue samples obtained in Italy in the past 10 years.
- HPV prevalence in 4,000 young women 18-24 y

All the studies used the same methodology and involved 10 different regions, so the obtained data can be considered representative of the entire national territory. Typing was performed using GP5+/GP6+ PCR primers that amplify a broad spectrum of HPV genotypes by targeting a 150-bp fragment within the L1 open reading frame (ORF) of the HPV genome. The preliminary data on HPV distribution in general population (18-60 years) showed a typical prevalence curve of HPV infection with the peak in the age group 21-24 with no difference statistically significant between North, Centre and South Italy. HPV 16, followed by HPV31, 51, 56, 58, 52 are the most prevalent HPV types in normal population (without lesions). In the study on CIN2, CIN3 and cancer tissue samples, cases were sampled through the electronic databases of the pathology units and paraffin embedded tissue samples were collected from historical archives according to a standardized protocol. To overcome any false negatives due to inhibitors commonly present in formalin-fixed paraffin-embedded tissue, all samples HPV negative at this first step, were retested diluted. To overcome misclassification of the HPV genotype resulting from potentially degraded DNA in aging archival paraffin-embedded tissues, on the samples that still remain negative for HPV we used a second PCR-based strategy amplifying a shorter DNA HPV fragment. The proportion of invasive cancers due to HPV 16 or 18 decreases with age at diagnosis. There was a statistically significant decreasing trend of HPV 16-18 proportion with age in invasive cancers passing from 92% in women <35 to 73% in women >55.

Conclusion: Despite southern Italy has a lower cervical cancer incidence than northern Italy, the prevalence in general population of high-risk infection is slightly higher. This suggests a situation in rapid transition probably due to changes in sexual behaviors.

SS 25-6

PREVALENCE OF TYPE-SPECIFIC HPV INFECTION IN WOMEN IN FRANCE BY AGE AND CERVICAL CYTOLOGY/HISTOLOGY

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Objectives: To assess HPV prevalence and genotype distribution, by age and cervical cytology/histology, among women undergoing routine gynaecological examinations.

Methods: Liquid-based cytology samples were tested for HPV DNA (Hybrid Capture® 2 [HC2]), HPV RNA (APTIMA®) and HPV genotype (Papillocheck®). Women with a cytology result of ASC-US or greater, or at least one positive HPV test were referred for colposcopy; abnormal colposcopies were confirmed by punch biopsy or LEEP.

Results: Of the 5,002 women included in the study, 515 (10.3%) were aged <25 years and 4,487 (89.7%) ≥25 years. HC2 testing revealed 755 HPV-positive women (15.1%); HPV genotyping identified 804 HPV-positive women (16.1%). HPV prevalence (HC2) was highest in women aged <25 years and 25–34 years (23.5% and 22.2%, respectively. Infection with more than one HPV type was detected in 9.3% of women aged <25 years and 5.2% of women aged ≥25 years. The prevalence of infection with at least one of HPV 6/11/16/18 was 5.8% (30/515) in women aged <25 years and 3.3% (147/4,487) in women aged ≥25 years. The most common genotypes were HPV 42 (5.6%), 51 (4.9%) and 16 (4.3%) among women aged <25 years and 16 (2.3%), 51 (2.1%), 42 (1.9%) and 53 (1.9%) among women aged ≥25 years. HPV prevalence (HC2) in women with normal cervical cytology was 19.0% and 10.4% in those aged <25 years and ≥25 years, respectively. There were no obvious differences in the proportions of HPV-positive results (HC2 versus genotyping). HPV prevalence (any type) increased with cytological severity. HPV 16 and/or 18 was involved in 33.3% (2/6) of HSIL in women aged <25 years and 46.5% (20/43) of HSIL in women aged ≥25 years. CIN3 or worse (CIN3+) was diagnosed in 29 women (2 aged <25 years, 27 aged ≥25 years). HPV prevalence increased with histological severity. HPV 16 was most strongly associated with a diagnosis of CIN3+ (OR 10.414 versus HPV 16 absent, P < 0.001).

Conclusions: The results indicate that almost all young women could benefit from HPV vaccination, but confirm the need for continued cervical screening in women over 25 years.
Background: Monitoring the prevalence of specific HPV types in the general female population is useful for evaluation of the potential effect of mass vaccination on the circulation of HPV vaccine types (effectiveness) or non-vaccine HPV types (cross-protection or type replacement). Enrolment strategies must result in samples that are consistently representative for the general population. Sweden, Denmark, Norway and Iceland have population-based cervical screening programs. Similarly, the HPV typing methodology needs to be comprehensive, internationally comparable and consistent over time. The WHO HPV LabNet Global Reference Laboratory (GRL) HPV typing method, based on broad general primer PCR with typing using Luminex and sequencing (J Clin Microbiol. 2009 ;47:541-6.) is accredited according to ISO15189 and has a defined sensitivity traceable to international standards.

Objectives: To monitor the prevalence of specific HPV types in the general female population in four Nordic countries.

Methods: Overall, 16550 randomly selected women <50 years of age, participating in cervical screening in 4 Nordic countries in the pre-vaccination era (2004-2006) were enrolled. HPV typing of their liquid-based cytological sample was centralized to the GRL and to date completed for 8607 women.

Results: Less than 1% of women were not enrolled because of declining informed consent, resulting in that the study has high representativeness for the general population. The overall HPV positivity for 40 genital types was 54.5% (95% CI: 52.7% - 56.0%) in the age group 18-26 and 22.4% (95% CI: 21.3% - 23.6%) in the age group 27-50. The prevalences varied between countries from 48%-61% in the younger age group and from 20%-25% in the older age group. The most common type in all countries and both age groups is HPV16, followed by HPV31, 52, 42, 56, 51, 18, 39, 66, 45 and 6 in order of decreasing prevalence.

Conclusions: The pre-vaccination baseline of population-based type-specific HPV prevalences has been assessed in 4 Nordic countries. HPV monitoring strategies with comprehensive HPV typing targeting young women participating in organised cervical screening is feasible.
PREVALENCE AND DISTRIBUTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS IN A SCREENING POPULATION IN GREECE

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Objective: Prevalence and type distribution of high-risk HPV in a female screening population across Greece, based on examinations of cervical samples, which were collected by local physicians and nurses in hospitals and health centers across the country.

Methods: Cervical samples from 4,667 women attending for cervical cancer screening have been examined using Hybrid Capture 2 (HC2) & Restriction Fragment Length Polymorphism - Polymerase Chain Reaction (RLFP-PCR).

Results: Almost six percent (5.8%) of women tested positive in HC2. The most common type was HPV16 (1.9% in the whole sample and 32.7% of the typed samples), followed by HPV53 (0.7 and 12.6%, respectively), HPV31 (0.7 and 12.2%, respectively), HPV35 (0.6 and 11.5%, respectively), HPV18 (0.4 and 7.4%, respectively), HPV51 (0.4 and 7.0%, respectively) and 22 more types. Almost 14% of the typed samples showed a coinfection with two HPV types and 2.1% with three types. There was a bimodal distribution by age, with the highest peak in women 20–29 years old and a lower peak in women 50–59 years old. Apart from the types originally included in HC2 cocktail, PCR analysis identified 15 more types (HPV6, HPV11, HPV34, HPV37, HPV38, HPV42, HPV53, HPV54, HPV55, HPV56, HPV 58, HPV61, HPV62, HPV66, HPV73, HPV82, HPV83). Eleven percent of HC2-positive results arose from single-type infections with HPV53 (10%) and HPV66 (1%), which are potentially high-risk types.

Conclusions: The prevalence of HC2 positivity in the largest Greek screening sample to date is 5.8%. The age distribution seems to follow a bimodal pattern. HPV16 is the most common type by far, followed by HPV 53, 31, 35, 18, and 51. Together with its related types, HPV 16 accounts for more than half of high-risk HPV infections. Surprisingly, the second most common type is HPV 53, which is more often detected in America than in Europe1 and its oncogenicity is questionable.


GENOTYPING PREVALENCE BY COUNTRY

de Sanjose Silvia1,2 on behalf of the RIS HPV TT3 study group

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Background

Human Papillomavirus (HPV) genotype distribution in invasive cervical cancer (ICC) may vary by country or world region. Histological confirmed ICC cases from 38 countries were assembled. HPV detection was done by polymerase chain reaction using SPF-10 broad-spectrum primers followed by deoxyribonucleic acid enzyme immunoassay and genotyping by reverse hybridization line probe assay (LiPA25) (version 1). Of 10,575 ICC cases, 8,977 were HPV-DNA positive (84.9%). The most common types were HPV 16, 18, 45, 33, 31, 52, 58 and 35, with a combined worldwide relative contribution of 91.3% (95% confidence interval (CI)=90.7%-92.0%). All world regions showed to have as most prominent types HPV 16 and HPV 18. When the eight most common types were selected in each region, the same types were identified with slight variations in their ranking.
CONSERVATIVE MANAGEMENT OF MICRO-INVASIVE CERVICAL CANCER

Michel Roy MD, FRCS

By definition, a “micro-invasive” cervical cancer is a lesion which is not detected with the naked eye, measures 7 mm or less in length and has a depth of invasion of 3 mm or less. The definition of a “micro-invasive” adeno-carcinoma of the cervix is identical but less generalized.

The diagnosis cannot be made on a biopsy, but only after a conisation or a hysterectomy. Therefore a diagnostic conisation must be performed to rule out frank invasion when a biopsy shows a “micro-invasion”.

The treatment can be conservative in young patients. A conisation can be “therapeutic” when margins are free of cancer and no vascular space invasion (VSI) is demonstrated. Radical hysterectomy is not indicated with stage Ia1. When VSI is found, there is controversy: radical surgery and pelvic lymphadenectomy versus simple hysterectomy.

SHOULD THE NEODJUVANT TREATMENT (CHEMOTHERAPY OR CHEMORADIOThERAPY) OF ADVANCED STAGE ADENOCARCINOMA BE SIMILAR TO SQUAMOUS CELL CARCINOMA?

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Cervical cancer is the second most common cancer among women worldwide and is the main cancer affecting women in sub-Saharan Africa, Central America and south-central Asia. In countries where effective (intensive national) screening programmes have been implemented for some time (North America, parts of Europe, Australia and New Zealand) there has been a significant decline in incidence of cervical cancer and associated mortality. This declining incidence is accounted for by a decrease in cervical squamous cell carcinoma (SCC), whereas the incidence of adenocarcinoma has risen or remained stable. Moreover, cervical adenocarcinoma is known for its unfavorable outcome, and it remains controversial whether this is due to a late detection by the cervical Papanicolaou test or poorer response to radiotherapy when compared with SCC and inclusion of subtypes (such as clear cell carcinoma, which is known for its dismal prognosis). Therapeutic modalities of cervical carcinomas depend on tumor stage and size but not on the histologic type. Several authors report a poor prognosis of adenocarcinoma of the uterine cervix because of their premature dissemination and inferior response to the radiotherapy. In contrast, other authors suggest that pure adenocarcinoma are not more radioresistant than SCC but that the dismal prognosis is related rather to the poorer tumor differentiation and a larger tumor size.

Concomitant neoadjuvant chemoradiation [concurrent cisplatin (Cis) and external-beam radiation (RT)] represents the standard for advanced stage cervical cancer (FIGO stage IB2–IVA), since 1999. This modality is superior to radiotherapy alone for local control, metastasis rate, disease-free and overall survival. A meta-analysis was recently performed, based on 18 trials with individual patient data, collecting a total of 3452 patients. Cisplatin-based chemotherapy was used in 85% of the patients. The results demonstrated a 6% improvement in absolute 5-year survival (from 60% to 66%) and 8% improvement in 5-year disease-free survival with chemoradiotherapy. The effect was consistent in patient subgroups defined by age, tumor histology, grade and whether or not pelvic nodes were involved. However, there was the suggestion of variation in the size of the benefit by tumour stage, with smaller benefits for patients with more advanced tumour stage. Patients with advanced stage IB2–IIA/B may benefit more from chemoradiotherapy than
patients with stage III and IVA, translating to a 5-year survival benefit of 10% for women with stage IB–IIA, 7% for women with stage IIB and 3% for women with stage IIIB–IVA.

Non-platinum-based regimens for chemoradiation appear to be as efficient as platinum-based chemotherapy. The most common regimen, however, is cisplatin monotherapy 40 mg/m² on a weekly schedule. A larger benefit was seen in two trials in which chemotherapy was given after chemoradiotherapy with an absolute improvement of 19% at 5 years. But the role of adjuvant chemotherapy after concomitant chemoradiation remains unclear and should be included in further clinical investigations.

Chemoradiotherapy increases acute toxicity, particularly gastrointestinal and haematological side-effects. Late effects of this combined treatment have not been extensively studied in the published literature.

Meanwhile series of trials tried to define the role of new agents combined with cisplatin and radiation therapy for locally advanced cervical cancer. The potential role of biologic agents in combination with chemoradiotherapy was also examined. The results were inconclusive and concurrent cisplatin (Cis) and external-beam radiation (RT) remains the standard therapy.

In ASCO 2009 it was presented a multicenter, open-label, randomized, phase III trial with the addition of gemcitabine (Gem) to Cis-RT compared with Cis-RT in 515 patients with bulky stage IIB to IVA cervical cancer. This trial showed the benefit of adjuvant chemotherapy with cisplatin–gemcitabine after concomitant chemoradiation, at the expense of increased but reported acceptable toxicity.

Yes or ... Maybe not! Unfortunately, there are very few credible and conclusive clinical data available, concerning different approaches to advanced stage cervical cancer, based on histologic differences (adenocarcinoma versus squamous cell carcinoma).

Concomitant neoadjuvant chemoradiation remains the standard and we may say that its introduction into routine clinical practice for locally advanced carcinoma of the cervix was one of the success stories of the Gynecologic Oncology.

SS 26-3

SHOULD ADENOCARCINOMA OF THE UTERINE CERVIX BE TREATED SIMILARLY TO SQUAMOUS CELL CARCINOMA?

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It is still a generalized believe that adenocarcinoma of the uterine cervix (ADC) represents a higher risk entity compared to the squamous cell carcinoma (SCC).

It has been demonstrated that, in the general incidence of invasive carcinoma of the cervix, ADC is progressively raising. (Data referred to the pre-HPV vaccine era).

But this epidemiological data, is only showing the cytological difficulties to detect ADC when it appears in absence of a concomitant squamous cell component.

In a previous study we analyzed the behaviour of adenocarcinoma in situ (AIS) according to the performed treatment. We registered 52 ADC and 11 AIS.

The mean age of the ADC group was 44.1 years and 40.1 for the AIS group.

72% of the AIS where initially treated by conization (8/11); we encountered a 25% affected resection margins after LLETZ. 5 post-conization hysterectomies were performed and we didn’t find residual disease in none of them. We found a vaginal VAIN III recurrence in a patient initially treated by hysterectomy.

We consider a conservative management of AIS in women who desire to preserve their fertility as a initial and adequate option.

We would also recommend to proceed with a laser conization. Tagloring size, shape and height of the cone with microcolplhoscopy can be of some help. Straight cold knife conisation is also an option. Negative resection margins will need strict follow-up. In a positive resection margins scenario, a new conization is mandatory.

If the patient doesn’t wish to preserve her fertility, hysterectomy is a reasonable option and, obviously, a more radical one. Nevertheless, due to the ethiology and natural history of the HPV related lesions of the lower genital tract, this radical option must be questioned.
Several different histological subtypes of cervical adenocarcinoma (AC) exist, with little practical relevance, however. Like its squamous cell counterpart (SCC), cervical AC develops through precursor lesions known as cervical glandular intraepithelial neoplasia (CGIN). The natural history of CGIN is not well established, with exception of the immediate precursor AIS (adenocarcinoma in situ). Even in the era of effective organised screening programs, early detection of AC and its precursors remains an enigma. In contrast to decreasing incidence rates of SCC seen in many countries, there is evidence suggesting that AC is in fact increasing in parallel. Whether this increase is real, however, remains a matter of continuous debate. In the earlier literature, it was constantly stated that cervical AC comprises some 5% of cervical carcinomas. While reviewing the literature between 1922 and 1947, Helper et al. (1952) recorded 15.476 cervical malignancies, of which 679 (4.5%) were ACs. According to another review covering the reports of 10.790 cervical malignancies during 1933-1978, an almost identical proportion (5.3%) of AC was found (Griffin et al., 1995). However, since the late 1970's, an increasing number of reports have appeared, where this proportion is considerably higher. The data from 73 studies covering the period 1919-1991 and comprising 6.222 cervical ACs was reviewed by this author, translating to the mean relative proportion of 9.3% (range from 3% to 34%) (Syrränen et al., Wiley, 2000). In Finland, the relative proportion of AC was 7.1% in the period 1956-1972, 9% in 1970-1974, and as high as 21.6% in 1976-1980. Interpretation of these data is not straightforward, however. Importantly, it should be considered, to what extent this relative increase in AC reflects 1) real increase in its incidence, 2) decrease in the incidence of SCCs, or 3) merely changes in the histological classification during these years. Most likely, all these factors may be contributory, with their impact varying from target population to another. However, there are several population-based studies confirming a genuine (two- threefold) increase in cervical AC over time, especially among women younger than 35 years, e.g. in the U.S., Canada, UK, Sweden, Norway, and Australia. Because no such increase has been seen in older age groups, it would argue against any changes in the classification practices as a source of bias. Taken together, the true nature of this observed increase of AC among young women must still be considered as a controversial issue. Likewise, the problems related to effective early detection of AC and its precursors (CGIN, AIS) remain to be solved.

### SS 26-5

**UNUSUAL ENDOCERVICAL ADENOCARCINOMAS: HPV DETECTION AND IMMUNOHISTOCHEMICAL ANALYSIS**

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**Objectives:** Endocervical adenocarcinomas of the usual type are etiologically related to infection with oncogenic HPVs. These tumors are typically positive for p16 and CEA immunostains. The goal of our study was to determine HPV status and immunohistochemical profiles of unusual subtypes of endocervical adenocarcinoma.

**Methods:** The study consisted of 26 cases of unusual subtypes of adenocarcinoma including clear cell (CCC, n=9), gastric (GAS, n=11), minimal deviation (MDA, n=3), mesonephric (MSN, n=1), serous (SER, n=1) and malignant mixed Müllerian tumor (MMMT, n=1). The control group consisted of 5 cases of usual endocervical adenocarcinoma (UEA). All cases were tested for HPV using SPF-10 PCR-LiPA, and immunostained for p16, HIK1083, hepatocyte nuclear factor 1-beta (HNF1β), p53, CEA, ER and PR.

**Results:** HPV DNA was not detected in any of the unusual adenocarcinoma subtypes with the exception of single case of SER in which HPV16 was detected. p16 positivity did not correlate with HPV status since 68% of HPV-negative tumors demonstrated p16 overexpression. p16 positivity was uncommon in GAS-MDA. HIK1083 positivity was limited to GAS-MDA. HNF1β was positive in majority of CCC but also in other tumor variants and UEA. CEA was consistently negative in CCC and in a single MSN, but positive in GAS-MDA, SER and UEA. p53 was diffusely positive in almost half of the GAS cases, while other variants showed focal or negative staining. PR was negative in all variant cases as well as all UEA. ER showed focal staining in rare cases.

**Conclusions:** Unusual variants of endocervical adenocarcinoma are not related to infection with HPVs and p16 overexpression in non-UEA does not correlate with HPV status. Negative staining for PR/ER may serve as a general marker of endocervical neoplasia. GAS-MDA may be differentiated from other adenocarcinomas with either positive HIK1083 stain or negative p16 stain; positive CEA stain differentiates GAS-MDA from CCC and negative PR/ER stains distinguishes GAS-MDA from benign endocervical glands. CCC may be distinguished from all other adenocarcinomas, except MSN, with negative CEA stain. Strong p53 positivity in SER may be useful in differentiation from UEA. MSN may be identified with negative CEA and ER/PR stains.
**PROPHYLACTIC VACCINATION OF OLDER WOMEN**

Harper DM

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**Objectives:** An important individual health benefit, not supported by public health authorities at this time, is the vaccination of older women who are in screening programs.

**Methods:** The incidence of CIN 2+ among women older than 45 years is estimated to be 385/100,000 women. While no one knows how quickly incident CIN 2+ in older women will progress to cervical cancer, clinical prudence indicates excisional treatment for this lesion. This incidence is equivalent to the combined incidence of vaginal, vulvar, anal and oropharyngeal cancers caused by HPV in women of all ages.

**Conclusions:** If vaccine protection is thought useful for these latter HPV associated cancers, then it is logical that vaccine protection would also be useful for older women, regardless of past HPV exposure, in countries with screening programs, where the true benefit of vaccination is the prevention of new CIN 2+.

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**MULTIPLE VERSUS SINGLE HIGH RISK HPV INFECTION IN YOUNG MALE AND FEMALE STI-CLINIC ATTENDEES IN THE NETHERLANDS**

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**Objectives:** Human papillomavirus (HPV) infection is a frequently occurring sexually transmitted infection (STI). The Netherlands have implemented vaccination against HPV type 16/18 for 12-year old girls and in March 2009 a catch-up campaign took place among girls aged 13-16 years. The aim of this study was to perform a baseline measurement, prior to the rollout of the catch-up campaign, to determine type-specific high risk (HR) HPV prevalences and potential risk factors for HR HPV, as well as single and multiple HR HPV infections, stratified by gender.

**Methods:** Data was cross-sectional collected in 12 STI clinics nationwide in The Netherlands among heterosexual males and females (respectively n=430, n=1136) aged 16-24 years. Self-collected vaginal or penile swabs were analyzed by PCR using SPF10 primers and LiPA genotyping. Co-infection with other STIs was determined by routine STI screening and data on sexual risk factors were collected. Genotype specific prevalences for HR HPV infections were determined and univariable and multivariable logistic regression analyses were performed to determine risk factors for single and multiple HR HPV infections.

**Results:** HR HPV was present in 40% (170/430) of males and 58% (661/1136) of females. Among the infected males and females, respectively 42% (71/170) and 47% (309/661) had multiple HR types. The two most prevalent HR HPV genotypes were HPV-51 and HPV-16.

Univariable and multivariable analyses of multiple HR HPV infections showed stronger relations with sexual risk behaviour in comparison with a single HR HPV infection. Independent risk factors for multiple HR HPV infection were: female gender (AOR:3.8, 95%CI:2.6-5.6), 5 – 9 sex partners (AOR:1.9, 95%CI:1.3-2.8) and 10 or more sex partners lifetime (AOR:4.2, 95%CI:2.7-6.7), 4 or more sex partners in the past 6 months (AOR:2.1, 95%CI:1.2-3.6), a history of Chlamydia or Gonorrhoea (AOR:3.2, 95%CI:2.0-5.2) and a current Chlamydia infection (AOR:1.7, 95%CI:1.0-2.6). Stratification for gender showed number of sex partners lifetime and a history of Chlamydia of Gonorrhoea as associated factors for both genders, condom use in combination with having or not having a casual or steady partner was also a determinant for HR HPV in females.

**Conclusions:** Females were more often infected with a HR HPV infection than males and in almost half of all infected cases multiple HR types was present. Multiple HR HPV infections were stronger associated with sexual risk behaviour than single HR HPV infections.
THE PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) INFECTION AMONG KOREAN PREGNANT WOMEN AND TRANSMISSION RATE OF HPVS TO THEIR INFANTS

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Objectives: To assess the prevalence of human papillomavirus (HPV) infection among pregnant women and to evaluate the rate of vertical transmission of HPVs to their infants.

Methods: One hundred ninety pregnant women and their infants delivered at Cheil General Hospital & Women’s Healthcare Center were prospectively recruited for this study between February 2010 and November 2010. Cervical swabs and blood samples were collected from the women at 32-36 weeks of gestation. Neonatal buccal swabs and cord blood were taken immediately after birth. HPV positive neonates were rechecked HPV DNA at 6 months postpartum. HPV genotyping with HPV DNA chip (MyGene Co., Seoul, Korea) was used to detect the HPV of mothers and neonates. Type specific PCR was performed to see HPV DNA in the maternal and cord blood in cases of mother and infant infected same types of HPV DNA.

Results: HPV DNA was positive in 19.5% (37/190) of mothers and 4.7%(9/190) of neonates. The rate of vertical transmission of HPV to their infant was 24.3%(9/37). HPV DNA type-specific maternal/neonate concordance was 100%. All 9 HPV positive infants were delivered vaginally. All HPV positive neonates were converted HPV negative at 6 months after birth. There was no viremia in maternal and cord blood in cases of mother and infant infected same type of HPV DNA.

Conclusions: Prevalence of HPV DNA in neonates born from HPV positive mothers was significantly high. However, these data suggest that neonatal HPV DNA positive is not true vertical transmission.
PREVALENCE AND RISK FACTORS FOR HPV INFECTIONS TYPES 16/18/45 IN A FRENCH COHORT OF 2 166 WOMEN AGED 14-23

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Objectives: Due to mass vaccination programs, investigation on prevalence and risk factors for HPV infection is the basis to assess prophylactic strategies against cervical cancer especially for young women concerned with HPV vaccination. The aim of this cross-sectional study was to assess prevalence and risk factors for high-risk human papillomavirus (HR HPV) types 16, 18 and 45 infection among a cohort of sexually active young women eligible for catch up vaccination in France.

Methods: Between 1997 and 2007, in the department of gynaecology, 2166 sexually active women aged from 14 to 23 years, were screened for HR HPV infection using the HC2 test (Qiagen). HR positive samples were further tested with the HPV16/18/45 Probe Set (Qiagen). Potential risk factors were investigated through a questionnaire dealing with education, marital status, sexual behavior, gynecological and obstetrical history, smoking intake and past STD sent to all women. The prevalence was age-adjusted using population data. High risk HPV DNA was detected in 44.5% women (966/2166) but only 17.9% of young women were positive for HPV16, 18 and/or HPV45. The age-adjusted prevalence of HPV16/18/45 infection was 17.3%. A total of 491 questionnaires were returned. Univariate analysis (only age-adjusted) identified potential predictors of HPV infection: like having more than two lifetime partners (OR: 6.09; 95% CI: 2.67-13.89), and cigarettes smoking (OR: 1.90; 95% CI: 1.31-2.76), while having a baby showed a protective effect (OR: 0.49; 95% CI: 0.26-0.94). Multivariate analysis, using regressive logistic in order to confirm the predictive role of the factors on HPV infection, will also be presented.

Conclusions: These findings constitute baseline data of HPV infection among young women concerned with HPV vaccination recommendations in France, which could be extended to young women whatever their sexual habits.

STUDY OF PREVALENCE OF HPV IN WOMEN WITH NEGATIVE CYTOLOGY

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HPV cervical infections have great variation between regions, which can be correlated with cervical cancer. Most of the infections are acquired when women begin sexual activity, becoming asymptomatic. There clearance is due to an effective immune response.

Objective: Prevalence of HPV types in women from 6 Health Units from Santa Casa Misericórdia Lisboa, with negative cytology.

Methods: 390 women with negative cytology where included in this study, with an average age of 29.6 years (range 20-62 years). Samples were collected for PreservCyt Solution and processed in the ThinPrep processor. All slides were stained with Papanicolaou and evaluated according to the Bethesda 2001 ed, and quality control was performed. HPV was screened by an in house qPCR (SFP primers), and positive samples were typed using Inno-Lipa or Papillocheck.

RESULTS: Of the 390 samples analyzed 44.9% were positive for HPV. The most prevalent types were HPV 16 (21.1%), HPV 31 (4.6%), HPV 52, 56, 58 and 68 (2.9%). Other types were less than 2%. Comparing women HPV negative and women HPV positive, we didn’t observe any deviation regarding the vaginal flora (10.3 vs 12.5%), fungal infection (5.1 vs 3.0%), trichomonas infection (1.8 vs 2.3%), atrophy (2.8% vs 2.9%) or inflammation (12.6% vs 7.8%). Regarding the contraceptive method, the results are very similar in both groups: use of condom (20% vs 17%) and hormonal contraception (35% vs 41%). There is also no difference between the ages of sexual debut in both groups: 18.8 years (range 13-41) in HPV negative women vs 18.5 years (12-39 years) in HPV positive women.

Conclusion: We didn’t saw any significant difference between both groups in terms of cytological anomalies (deviation of the vaginal flora, fungal infection, trichomonas infection, atrophy or inflammation), age of sexual debut and contraceptive method used. Follow up of HPV positive women can give us more data to predict women that have higher risk for disease progression.
CERVICAL HPV INFECTION IN 18 YEARS OLD ITALIAN GIRLS

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Objectives: Cervical cancer screening programme in Italy starts at age 25. Little is known about the epidemiology of HPV before this age. At IEO a prospective observational study on cervical HPV infection in 18 years old girls from Milan undergoing 4HPV vaccination is ongoing. Before vaccination all the girls answered an epidemiological questionnaire.

Methods: The presence of HR-HPV was tested with Hybrid Capture II (HC2)(Qiagen). HC2 positive specimens were genotyped with LINEAR ARRAY (Roche Diagnostics). Cervical cytology was done only to girls that declared not to be virgo. Any positivity to the tests was further examined with colposcopy and biopsies, when needed.

Results and Conclusions: 533 girls were enrolled. 346 (64.9%) were never smokers and only 12 (3.5%) were HPV positive. 160 (30%) were actual smokers while 26 (4.9%) were past smokers, and between these 32 (20%) and 4 (15.4%), respectively, were HPV positive. 200 girls (37.5%) were virgins. Of these 6 (3%) resulted positive to HC2: 1 was positive to HPV16, 5 resulted negative to HPV when genotyped. 333 (62.5%) had already had sexual intercourses. Of these 42 (12.6%) were HC2 positive. 12 resulted positive to HPV16, 3 to HPV18, 18 to other HR-HPVs, 5 to low risk HPVs, while 5 were negative to HPV when their sample was genotyped. The rate of positivity to HPV was higher between girls having their sexual debut in earlier ages: 26.3% positivity when at 14 yrs old (5/19), 23.4% when at 15 yrs (15/64), 8.4% at 16 yrs (10/119), 7.1% at 17 yrs (6/84), 16.2% at 18 (6/37). The positivity to HPV increased with an increase in the number of sexual partners: 6.7% of positivity between girls with 1 partner (11/165), 5.8% between girls that had 2 partners (4/69), 14.3% with 3 partners (6/42), 36.4% with 4 partners (8/22), 47.8% with 5 or more partners (11/23). Cervical cytology was negative in 309 cases (92.8%), while in 9 (2.7%), in 14 (4.2%), and in 1 (0.3%) cases the result was respectively ASCUS, LSIL and ASCH. No CIN2+ was identified. The results show an overall low positivity to HPV in this population compared to other papers: however, the rate of HPV positivity was strictly related to the sexual life. The cytology result of LSIL was four times more frequent than in the screening population, while no CIN2+ was identified, confirming the indication to avoid screening at this age.

OCCURRENCE AND DYNAMICS OF HPV COINFECTIONS AMONG MONTREAL UNIVERSITY STUDENTS

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Objectives: Coinfections with multiple types of HPV are common, particularly among young, sexually active women. The objectives of this study were to examine the prevalence, incidence and duration of HPV coinfections as well as the risk of acquiring additional HPV types based on infection status at enrollment.

Methods: Participants came from the McGill-Concordia Cohort, a longitudinal study of the natural history of HPV infection in 621 female university students in Montreal, Canada. Recruitment occurred between 1996-1999 and women were followed for 2 years at 6-month intervals. At each visit, cervical specimens were collected for HPV DNA detection and typing using MY09/11 PCR and the Line Blot Assay. Two definitions of coinfections were used: cumulative coinfection over follow-up and concurrent coinfection at each visit. Person-time was used to calculate incidence and Kaplan-Meier techniques were used to estimate mean duration of infections.

Conclusions: 28% of the cohort had multiple HPV types detected at the same visit and 33% had multiple types detected over follow-up. Among HPV-positive women, these proportions were 53% and 62%, respectively. Virtually all HPV types were detected more often as coinfections than as monoinfections. The incidence rate of concurrent coinfection was 9.2 (95%CI 7.5-11.2) per 1000 person-months. Women with a HPV monoinfection at enrollment had nearly 3 times the risk of acquiring an incident coinfection compared to HPV-negative women: RR = 2.7 (95%CI 2.5-3.0). Compared to HPV-negative women, the RR of acquiring a new type of HPV was 1.8 (95%CI 1.6-2.0) among women with a HPV monoinfection at enrollment and 2.3 (95%CI 2.0-2.7) among women with a coinfection at enrollment. Coinfections generally increased the mean duration of individual and grouped type-specific infections. These results confirm the high prevalence of HPV coinfections among young women and suggest that coinfections are associated with increased acquisition of additional HPV types and greater infection persistence among young women.
Carcinoma of the cervix is the second most common type of tumour within the female population, and is one of the main causes of death worldwide. The occurrence of a persistent infection caused by Human Papillomavirus (HPV) is considered the main cause for the development of cervical cancer, especially if there are high risk HPV genotypes. Taking into consideration that Cytology has a sensitivity factor of 60% and a specificity of 95% and that genotyping represents values of 85% and 84%, respectively, several authors defend the use of both Cytology and Genotyping to accurately diagnose premalignant lesions of this tumour.

Objectives: Verify the relationship between cytological and histological diagnostics and the results obtained in the detection and typification of HPV in Pap smear samples with ASC-US, ASC-H, LSIL, HSIL and determined the frequency of different types of HPV that are associated with these diagnostics.

Methodology: The results of 59 Pap smears with biopsy and genotyping, from a private Lisbon Laboratory diagnostics, were analysed through a retrospective study. The Pap smears used in the study were performed using the liquid-based cytology method, Labonor® and manually screened and reported according to the Bethesda 2001, genotyping with the Clinical Arrays of Human Papilomavirus (Genomica®) and by sequential genotyping in the negative cases. Statistical analysis performed by the Chi-Square Test and also by frequency analysis.

Results: The detection of HPV, was present in 60% of the ASC-US cases, in 96.7% of the low grade intraepithelial lesions (LSIL) and in 100% of the high grade lesions (HSIL) The presence of HPV 16 was detected in 26.7% of the ASC-US diagnostics, and HPV 53 being associated to the ASC-US, LSIL and HSIL diagnostics. A significant statistical relationship (p < 0.05) was noted between the cytological and histological diagnostics and the genotyping of HPV.

Conclusion: Although the percentage of agreement between cytological and histological diagnostics was not 100% it was statistically significant. Therefore, the association of genotyping to Cytology and using Histology as a gold standard is a complementary method to the early detection of carcinoma of the cervix, thus reducing the mortality rate through this type of cancer.

Background: Limited data are available describing human papillomavirus (HPV) infection prevalence and genotype distributions among young women in Brazil. Such information is relevant regarding the potential impact of HPV prophylactic vaccination and HPV-based screening strategies for the country.

Objectives: This study aimed to determine the overall and type-specific prevalence of human papillomavirus infection among young female university students from a central area of Brazil.

Methods: Type-specific prevalence of HPV was investigated in 200 young female university students, aged 18–25 years, from 16 different cities of a central area of Brazil. Cervical scrape specimens were tested for HPV DNA detection by PCR, using consensus L1 PGMY09/11 primers. HPV genotyping was performed on all HPV positive samples using the Roche reverse line blot assay.

Results and Conclusions: Overall prevalence of HPV among young female university students was 47.0% (94/200). Infections with high risk HPV genotypes (HR-HPV) were very prevalent (77.7%; 75/94). The most common HPV genotypes detected in the group were HPV type 16 (21.3%), followed by HPV type 58 (12.8%), HPV type 31 and HPV type 66 (11.7% each), and HPV type 52 and HPV type 61 (10.6% each). Multiple infections were observed in the majority of the positive cases (54.2% 51/94). Our data provide baseline information for further prevention strategies of HPV infection.

This study was supported by FAPEG, Fundação de Apoio à Pesquisa do Estado de Goias.
PREVALENCE AND TYPE DISTRIBUTION OF HUMAN PAPILLOMAVIRUS IN HEALTHY JAPANESE WOMEN AGED 20 TO 25 YEARS OLD ENROLLED IN A CLINICAL STUDY

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Objectives: The HPV-16/18 AS04-adjuvanted vaccine (GlaxoSmithKline Biologicals) was recently shown to be effective and immunogenic with a clinically acceptable safety profile in Japanese women aged 20 to 25 years [1]. As information regarding HPV prevalence in this population is lacking, we analyzed the baseline data from that study and report here the prevalence rates of HPV infection in young healthy Japanese women.

Methods: 1040 Japanese women (20-25 years) were enrolled in a phase II, double-blind, controlled, randomized, multicenter study (NCT00316693). At study entry, cervical specimens were collected and tested by line probe assay for 25 HPV-types and by HPV-16/18-specific polymerase chain reaction (PCR).

Conclusions: The women were from 7 areas throughout Japan: 457 Tokyo (44.0%), 160 Aomori (15.4%), 136 Osaka (13.1%), 128 Fukui (12.3%), 91 Kagoshima (8.8%), 39 Hiroshima (3.8%) and 29 Miyazaki (2.8%). The most frequently detected HPV-type in baseline cervical specimens was HPV-52 (8.1%, 84 women); followed by HPV-16 (6.5%, 68), HPV-51 (4.5%, 47), HPV-18 (4.0%, 42) and HPV-31 (3.8%, 39). The proportion of HPV DNA-positive women increased with severity of cytological abnormalities: 26.1% (237/908) in normal cytology, 93.3% (70/75) in low-grade squamous intraepithelial lesion (LSIL) and 100% (7/7) in high-grade squamous intraepithelial lesion (HSIL). The relative contribution of HPV-16 and -18 was 4.1% (37/908) and 3.0% (27/908) for normal cytology cases, and 20.0% (15/75) and 16.0% (12/75) in LSIL. HPV-16 was found in four of seven HSIL cases (57.1%) and five of the six cases of cervical intraepithelial neoplasia (CIN) 2+ (83.3%). Multiple and single HPV infections were reported in 13.5% (140/1039) and 20.7% (215/1039) of all women.

This study is the first to nationwide evaluate the HPV prevalence for healthy young Japanese women aged 20 to 25 years, which is relevant to current Japanese vaccination practices. The HPV prevalence rates reported in this study underline the importance of HPV vaccination at a young age and should be useful for monitoring changes in HPV prevalence after widespread HPV vaccination in Japanese women


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HPV TYPE DISTRIBUTION IN A COHORT OF 856 FRENCH WOMEN WITH ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE (ASCUS) CERVICAL SMEARS

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Introduction: Data on HPV type distribution are essential for estimating the impact of HPV vaccines on cervical cancer and cervical screening programs. We report HPV genotype distribution in cervical smears with ASCUS, coming from a cohort of women and compare it to that reported in the EDITH I, II and III studies which investigated HPV genotypes in low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and squamous cervical carcinoma histological specimens.

Methods: Between January 2007 and December 2009, 856 cervical smears with a diagnosis of ASCUS have been collected (i) among women undergoing a routine medical check-up provided by a French National Health Insurance (n=272) and (ii) among women consulting in the gynaecology department of Tours University Hospital (n=169). HPV DNA was looked for using the INNO-LiPA HPV Genotyping Extra* (Innogenetics). Sequencing of L1 region was performed when HPV was untypable.

Results and conclusion: Overall prevalence of LiPA detectable HPV was 51.5%. There was no age difference between women tested HPV positive or negative. Mono-infections with high- (HR) and low-risk (LR) HPV, were found in 230 and 37 samples, respectively. Type 16 was the most prevalent (16%), followed in order of frequency by type 51, 70, 52, 53, 31, 44, 66 and 35. Co-infections with presence of HR and LR HPV were found in 103 samples (23%). Type 52 was the most prevalent (45%). Overall, HPV 52 was the most prevalent (23%) followed in order of frequency by HPV 16, 54, 31, 51, 39, 68, 53, 70. Untypable HPV were detected in 41 samples (17%).

HPV 16 prevalence increased with the severity of lesions (16% in ASCUS smears but 73% in cancer) confirming its high oncogenic potential. Only four HPV type (16, 31, 52, 68) were found in ASCUS as well as in LSIL, HSIL smears and invasive cancers. Although their prevalence in cervical cancers is low (for instance, 3% for type 52), these HPV should be present in screening tests.
THE EPIDEMIOLOGICAL BURDEN OF HPV-RELATED CANCERS IN MEN IN EUROPE: A COMPARISON TO WOMEN

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Objectives: To estimate the epidemiological burden of HPV-related cancers in men in Europe and to compare it to the burden of HPV-related cancers in women.

Methods: All cancer sites potentially related to HPV in men were identified. The annual number of new cancer cases potentially related to HPV in men in Europe was estimated based on cancer incidence rates extracted from IARC’S CI5, Vol. IX (data available for 26 European countries) and on the 2008’s Eurostat population. The burden specifically attributable to HPV was then evaluated by applying cancer-specific HPV prevalence estimates extracted from the most relevant published data available. Estimates in men were then compared to similarly obtained estimates in women.

Results: Expected mean annual number of new cancer cases in sites potentially related to HPV by gender in Europe (2008 basis)

<table>
<thead>
<tr>
<th>Cancer sites</th>
<th>Expected number of new cancer cases irrespective of HPV status</th>
<th>Expected number of new cancer cases attributable to HPV (all types)</th>
<th>Expected number of new cancer cases attributable to HPV 16/18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>-</td>
<td>30,157</td>
<td>-</td>
</tr>
<tr>
<td>Vagina</td>
<td>-</td>
<td>1,795</td>
<td>-</td>
</tr>
<tr>
<td>Vulva</td>
<td>-</td>
<td>7,106</td>
<td>-</td>
</tr>
<tr>
<td>Head and neck</td>
<td>63,534</td>
<td>12,957</td>
<td>13,301</td>
</tr>
<tr>
<td>- oral cavity</td>
<td>18,915</td>
<td>6,918</td>
<td>3,026</td>
</tr>
<tr>
<td>- oropharynx</td>
<td>19,197</td>
<td>3,294</td>
<td>4,860</td>
</tr>
<tr>
<td>- larynx</td>
<td>25,422</td>
<td>2,745</td>
<td>5,415</td>
</tr>
<tr>
<td>Anus</td>
<td>2,027</td>
<td>3,554</td>
<td>1,709</td>
</tr>
<tr>
<td>Penis</td>
<td>2,907</td>
<td>-</td>
<td>1,356</td>
</tr>
<tr>
<td>Total</td>
<td>68,468</td>
<td>55,569</td>
<td>16,366</td>
</tr>
</tbody>
</table>

Conclusions: The overall burden of HPV-related cancers in men is high in Europe. Among all new annual cancer cases attributable to HPV16/18, around 30% were estimated to occur in men. Beyond cervical cancer, the estimated burden of HPV-positive cancers is more common in men than in women and is mainly driven by Head and Neck cancers. As in women, the vast majority of HPV positive cancers in men is related to HPV types 16 and 18 and may thus be potentially prevented by HPV vaccination.

A MULTI-CENTER STUDY OF HPV16 & 18 PREVALENCE IN WOMEN? 30 YEARS OF AGE USING THE CERVISTA® HPV 16/18 TEST

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Objectives: To determine the prevalence of HPV16 & 18 in women ≥ 30 years of age in a routine screening population representing 3 geographically diverse areas using the Cervista® HPV 16/18 test.

Methods: Cervista HPV 16/18 testing was performed on 1,481 residual ThinPrep® cervical cytology samples previously tested using the Cervista HPV HR test. Based on the 2001 Bethesda System Classification, the sample population consisted of 1402 NILM, 57 ASC-US, 20 LSIL and 2 HSIL. One hundred twenty nine (8.7%) of the 1,481 samples were positive for HPV HR and 1,352 (91.3%) of the samples were negative. HPV16 & 18 prevalence was stratified by cytology category, age and HPV HR status.

Conclusions: The overall prevalence of HPV16 & 18 in women ≥ 30 years of age in the population tested was 2.4% (35/1481) for HPV16, 0.2% (3/1481) for HPV18 and 0.0% (0/1481) HPV16 & HPV18. In the NILM population, the prevalence of HPV16 & 18 was 2.1% (29/1402) and 0.1% (1/1402), respectively. The prevalence of HPV16 in the NILM population decreased with increasing age from 2.6% in the 30 to 39 year age group to 2.3% for ages 40 to 49 years and to 1.1% for ≥ 50 years of age. No relationship between HPV18 and age could be determined in the NILM population because only one sample, which was in the 30 to 39 year age group, was positive for HPV18.

Of the 129 samples in the overall population that were positive for HPV HR, 30 (23.3%) were positive for HPV18 and 35 (27.3%) were positive for HPV16. Among the 1,352 HPV HR negative samples, 1,344 (99.4%) were negative for HPV 16 or 18 and 8 (0.6%) were positive for HPV16 or 18.

This data demonstrates the prevalence of HPV16 & 18 in a screening population as determined by the Cervista HPV16/18 genotyping test. Detection of HPV16 & 18 in conjunction with HPV HR testing and cervical cytology may be used to guide patient management in women ≥ 30 years of age.
HPV DNA/MRNA TESTING AND SMOKING AS A RISK FACTOR IN PREDICTING HIGH GRADE CERVICAL DISEASE IN WOMEN PRESENTING WITH LOW GRADE CERVICAL LESIONS


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Background: High Risk (HR) HPV infection is identified in up to 80% of women with LSIL, most of which regress spontaneously. Thus co-factors are believed to be involved in the transition from transient to transforming HPV infections. This study evaluates the significance of smoking, through urinary cotinine analysis, and HPV infection in development of high grade disease. The study is performed under CERVIVA funded by the Health Research Board Ireland and the Irish Cancer Society.

Methods: Women presenting for their first visit to colposcopy at the National Maternity Hospital, Dublin, with LSIL and ASCUS were invited to participate. At enrolment women gave a urine sample for cotinine analysis, a smear specimen for HPV testing and a biopsy for histological evaluation. HPV DNA was detected by Hybrid Capture II (Qiagen, UK), HPV mRNA by the PreTect™ HPV Proofer (NorChip AS, Norway) and cotinine analysis by the Immulite 2000 Nicotine Metabolite assay (Siemens, UK).

Results: 618 women were HPV tested. Histology confirmation shows 21% (127/618) were CIN 1, 24% (147/618) were CIN 2+ and 52% (321/618) were normal. The overall prevalence of HR HPV DNA was 62% (383/618) compared with 38% (232/618) E6/E7 mRNA positive cases. The likelihood of being mRNA positive decreased with age (30-39 vs <30: OR=0.61, 95%CI 0.39-0.95; 40+ vs <30: OR=0.47, 95%CI 0.26-0.86). Of the 618 women 160 were treated by LLETZ. HPV prevalence rates in this cohort are 75% and 49% for HPV DNA and mRNA respectively. For detection of CIN 2+ lesions the sensitivity and specificity was 85% and 52% for HPV DNA and 58% and 80% for HPV mRNA. HPV mRNA was detected in 35% (113/318) of non-smokers (cotinine <50ng/ml) compared with 43% (95/223) of smokers (cotinine >50ng/ml).

Conclusion: HPV testing is a useful tool in the management of women presenting with low grade abnormalities. In addition it suggests that women who smoke are at increased risk of persistent HPV infection, associated with progression to high grade cervical lesions.

HPV DNA PRESENCE AND HPV GENOTYPES AS PROGNOSTIC FACTORS IN CERVICAL CANCERS


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Objectives: The aim of this study was to assess the prognostic potential of the presence of HPV DNA and HPV genotypes in cervical cancers.

Methods: From Jan 2008 to Dec 2010, medical records of 134 patients who were diagnosed with cervical cancers were retrospectively reviewed. We used an HPV DNA chip to detect the type-specific sequence of HPV from cervical swabs. The mean age of the patients was 48.5 years (28-76) and distributions of the stage were 46 (34.3%) in Ia1, 5 (3.7%) in Ia2, 52 (38.8%) in Ib1, 16 (11.9%) in Ib2, 6 (4.5%) in IIa1, 3 (2.2%) in IIa2, 5 (3.7%) and 1 (0.7%) in IIIa. The histological cell types were 91 (67.9%) squamous cell carcinomas, 34 (25.4%) adenocarcinomas, and 9 (6.7%) adenosquamous cell carcinomas. HPV-DNA of any type was not detected in 18 patients (13.4%) and 16 patients (11.9%) had multiple infections. HPV 16 was the most predominant type and was detected in 44.8% (60/134) of cases. Other types were HPV 18 in 13.4% (18/134), HPV 58 in 7.5% (10/134), HPV 31 in 4.5% (6/134) and HPV 35 in 3.0% (4/134), respectively. We divided the patients into four groups: HPV negative, HPV 16, HPV 18, and HPV others (except HPV 16 and HPV 18) and assessed the correlation with clinicopathologic characteristics. HPV 18 and HPV negative were more prevalent in cases of adenocarcinomas (p=0.000). However, neither presence of HPV DNA nor the HPV genotype was related with stage, lymph node metastasis, parametrial invasion, lymphovascular space invasion, tumor size, vaginal involvement and tumor marker.

Conclusions: These results show that neither the presence of HPV DNA or the HPV genotype was not associated with prognostic significance and HPV 18 and HPV negative were more prevalent in adenocarcinoma.
PROGNOSIS OF CERVICAL CANCER AMONG IMMIGRANT AND SWEDISH BORN WOMEN, DURING 1960 TO 2005


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Objectives: There are differences in the risk of invasive cervical cancer between immigrant and Swedish born women, largely explained by participation to cervical screening. In this study we investigate the prognosis after diagnosis of invasive cervical cancer for immigrant and Swedish born women, assessing socio-economic status, education, and adherence to the cervical screening program.

Methods: All women diagnosed with invasive cervical cancer in Sweden during 1960 to 2005 were followed up for death due to cervical cancer. Prognosis was assessed for immigrant and Swedish born women separately, and discerned by socio-economic status, education and adherence to the cervical screening program during 1993 to 2005. Hazard ratios of death due to cervical cancer within 10 years of follow-up (HR) were calculated with 95% confidence intervals (CI) in proportional hazard regression models.

Conclusions: No big differences in 10-year cause specific survival could be discerned between immigrant and Swedish born women (HR 0.9, CI 0.7-1.0). However, there were large differences in prognosis depending on participation to cervical screening both for immigrant and Swedish born women. HR was 0.2 for screen detected, compared to symptomatic, never screened, cases (CI 0.2-0.3), with no differences between immigrant and Swedish born women. For symptomatic, interval and overdue cases, with a Pap smear, there was a slightly lowered risk (HR 0.7 and 0.8, respectively), compared to women not having a Pap smear. Women with low manual socioeconomic status conferred a slightly worse prognosis, compared to high non-manuals, as did women with mandatory or college education, compared to university education. These patterns were quite similar for immigrants and Swedish born women. When it comes to prognosis of cervical cancer, there seem to be no large differences between immigrant and Swedish born women, while attendance to cervical screening is the main determinant of prognosis. Socioeconomic status and education also may play a role in the prognosis of cervical cancer.

INFECTION WITH HIGH-RISK HPV GENOTYPES IN WOMEN UNDERGOING IN VITRO FERTILIZATION IN SLOVENIA

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Objectives: The aim of the present study was to establish the prevalence of high-risk human papillomavirus (hr-HPV) genotypes in Slovenian infertile women undergoing in vitro fertilization (IVF) program. Furthermore, we wanted to correlate the outcome of IVF treatment with HPV status.

Methods: Every woman who started hormonal treatment from January to the end of May 2010 was included in our study. Controlled ovarian hyperstimulation was achieved with recombinant gonadotrophins and GnRH agonists or antagonists. Two cervical smears were obtained from every woman; one for cytological analysis and one for hr-HPV detection. Specimens were tested with RealTime High Risk HPV test (Abbott Molecular Inc., Des Plaines, IL). A total of 195 women were included. Mean age was 33.7 ± 4.36 years. The mean number of retrieved oocytes was 7.7 ± 5.7; mean percentage of mature oocytes was 71.7 ± 28.15 and mean percentage of fertilized oocytes was 53.0 ± 29.48 per woman. Mean number of embryos was 4.0 ± 3.6 per woman; mean number of blastocysts was 1.1 ± 1.4 per woman. A total of 56/157 women with embryo transfer were pregnant; the pregnancy rate per embryo transfer thus being 35.7%. A total of 187/195 women (95.9%) had normal cytological smear; 6 (3.1%) had reactive changes, one (0.5%) had atypical squamous cells and one (0.5%) had mild dyskaryosis. A total of 179/195 women (91.8%) were hr-HPV negative and 16/195 women (8.2%) were hr-HPV positive; 3 women (1.5%) were infected with HPV 16, 2 women (1.0%) were infected with HPV 18 and 11 women (5.6%) were infected with other hr-HPV genotypes. Hr-HPV infection had no effect on oocyte and embryo quality or pregnancy rate.

Conclusions: A total of 8.2% of women included in an IVF program were hr-HPV positive, which is less than the average prevalence in women aged 30-35 years (~12%). Hr-HPV infection was not associated with the percentage of mature oocytes or the percentage of fertilized oocytes. Gynecological exam with colposcopy performed in HPV 16 and HPV 18 positive women revealed one case of cervical intraepithelial neoplasia grade 1.
HPV IN VULVAR CANCER – CAN ITS PRESENCE BE USED AS A PROGNOSTIC FACTOR?

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Objectives: Human Papilloma Virus (HPV)-dependent vulvar cancer often occurs in younger patients. The proof of HPV-DNA in lymph nodes in cervical cancer showed an increased risk for a fatal course of disease. Aims of the study were to investigate whether HPV-DNA could be detected in non-metastatic lymph nodes and its influence on tumor stage, disease-free- and overall-survival.

Methods: Surgical tissue of patients with squamous-cell-carcinoma of the vulva was collected. HPV-DNA was isolated via sensitive real time PCR. Follow-up data was obtained from patients’ records. Results were combined and compared with respect to initial extent of the disease and to HPV status. Histological samples were obtained from 40 patients. Patients with HPV positive (HPV+) tumors had significantly more advanced tumor stages following the TNM classification (p = 0.0046). None of the non-metastatic lymph nodes were HPV+. Average age of patients with HPV+ tumors was lower than the HPV negative (HPV-) tested patients (62.5 yrs versus 71.3 yrs, p=0.0569). There were no statistically significant differences in nodal metastatic spread and FIGO classification. More patients with HPV+ tumors suffered from recurrence or fatal course than in the HPV- group.

Conclusions: We found significant differences in tumor stages in HPV+ vs. HPV- tumors in vulvar squamous cell carcinoma. The group size was too small to identify more statistically significant results, nevertheless, a tendency towards more fatal courses in the HPV+ group was found. As this form often occurs in younger women, a controlled trial should be conducted, implementing a more aggressive adjuvant treatment to prevent early recurrence.

RISK FACTORS FOR PRE-CERVICAL CANCER IN HIV-POSITIVE PREGNANT WOMEN

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Background: Cervical cancer is the second most common type of cancer among women worldwide. The incidence of cervical intraepithelial neoplasia (CIN) is 4-5 times higher among HIV-infected than HIV-negative. Pregnancy is ideal to do a screening in those women.

Methods and Results: A retrospective review of 141 HIV-1 and 13 HIV-2 infected pregnant women attending in our department between January 2004 and December 2009 was undertaken. SPSS® 18.0 was used to do statistical analysis.

Thirty-nine percent (n=56) of patients had an abnormal cytology, all of them infected with HIV-1. All patients were referred for colposcopy according to our guidelines. From histological findings the prevalence of CIN was 11%.

In the group with abnormal cytology , the mean age was 30 years, the HIV transmission was sexual in 74% and 53% were under HAART in beginning of pregnancy. The mean viral load was 40668 (min <50; max 444000) and mean CD4 cells count was 360 (min 51; max 980) Associated with abnormal cytology coexist vaginal infection (syphilis, trichomoniasis, chlamydia or vaginosis) in 32 patients.

In the group with normal cytology , the mean age was 30 years, the HIV transmission was sexual in 77% and 30% were under HAART in beginning of pregnancy. The mean viral load was 30937 (min <50; max 594800) and mean CD4 cells count was 477 (min 44; max 1425) Associated with abnormal cytology coexist vaginal infection (syphilis, trichomoniasis, chlamydia or vaginosis) in 40 patients.

Conclusions: A history of immunosuppression, including decreased CD4 cell counts, and higher viral loads, is a risk factor for pre-cervical cancer in women with HIV. We found that antiretroviral therapy had no protective effect against pre-cervical cancer and other sexually transmitted infections were not associated with higher risk for pre-cervical cancer.
ATTENDANCE TO SCREENING AND RISK OF CERVICAL CANCER AMONG IMMIGRANTS IN SWEDEN, DURING 1993 TO 2005

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Objectives: Cervical screening has effectively reduced the incidence of cervical cancer in high income countries, including Sweden. Since the 1990s women are invited every 3 years at ages 23-50 and every 5 years at ages 51-60. In this study we investigate the degree of participation and effect of adherence to the cervical screening program on the risk of developing invasive cervical cancer, among immigrant and Swedish-born women, and to what extent adherence to screening modifies differences in risk of cervical cancer between Swedish-born and immigrant women.

Methods: Degree of participation to cervical screening was estimated for immigrant and Swedish-born women between 23 and 60 years from 1993 to 2005, stratified by age at migration. We also estimated incidence rates and rate ratios for women adhering or not to the cervical screening program. Finally, we assessed the effect of adherence to screening on the relative risk of cervical cancer for immigrant, compared to Swedish-born women.

Conclusions: The degree of participation according to recommendations was 61 % and 51% among the Swedish-born and immigrant women, respectively, with large variations among the immigrant groups. Degree of participation was lower with older age at migration and women who where non-adherent to the cervical screening program had a 5-fold risk of invasive cervical cancer, or more, compared to those who were adherent. However, screening adherence modified the relative risks of cervical cancer among immigrant, compared to Swedish-born women, only modestly. Although adherence to cervical screening drastically reduces risk of cervical cancer, it can not completely explain the differences in risk of cervical cancer observed between immigrant and Swedish born women. Immigration after age 40 to 50 is an important barrier for participation to cervical screening.

THE EFFICACY OF LIQUID-BASED CYTOLOGY IN PUBLIC SCREENING FOR CERVICAL CANCER IN SOUTHERN JUTLAND

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Objectives: The Southern Jutland Hospital of Sonderborg in Denmark serves a population of about 250,000 people. Public screening for cervical cancer in our area was implemented in 1979 and liquid-based cytology (LBC) was used since 2006. The efficacy of LBC in public screening for cervical cancer is apparently still a matter of dispute. The objective of this retrospective study was to compare LBC and conventional cytology regarding quality, rationality and the ability to detect high-grade lesions in public screening.

Methods: The study included a pre-LBC group of 62,709 conventional PAP-smears with the corresponding 941 cervical punch biopsies and 475 cone biopsies received in 2003–2005, and an LBC group of 67,925 liquid-based PAP-smears with corresponding 1,400 punch biopsies and 628 cone biopsies received in 2007-2009. The average age of the attending women in both groups was 33.5 years. One colposcopy was performed for every 66 and 48 PAP-smears in pre-LBC and LBC groups respectively, and one conisation for every 132 and 100 PAP-smear in the same order.

The detection rate of CINII+ in cytology increased from 1.1% to 2.7%, and the unsatisfactory rate dropped at the same time from 8% to 1.4% after the implementation of LBC. The rate of smears without endocervical cells dropped likewise from 7% to 1.7%. Four percent of the smears in both groups were repeat smears for ASCUS+. The number of cones increased by 32% and number of CINII+ cases in these cones increased likewise by 38% after the introduction of LBC. CINII+ lesions were thus detected in 81% and 85% of cone biopsies in the pre-LBC and LBC groups respectively. Smears that were reported benign in the last 4 years prior to conisations were counted in both groups without rescreening and without distinction between screening’s errors and sampling’s errors. In the pre-LBC group there were normal smears in 73 of the 475 cones (17.5%) and in the LBC group 56 of the 628 cones (9%).

Conclusions: The remarkable reduction of the unsatisfactory smears and smears without endocervical cells after the application of LBC illustrate the superiority of this system. The increase of CINII+ detection rate in cytology and the corresponding increase in number of colposcopies and cone biopsies that harboured at least the same rate of CINII+ is yet another advantage showing an increase in sensitivity without a reduction in specificity.
IMPROVING STRATEGIES FOR CERVICAL CANCER PREVENTION: THE IMPACT OF SUREPATH LIQUID-BASED CYTOLOGY IN BRAZILIAN REMOTE RURAL AREAS. PRELIMINARY RESULTS OF RODEO STUDY

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Objectives: To analyse the performance of SurePath liquid-based cytology (LBC) in women submitted to the gynaecological examination in mobile units (MUs) from Barretos Cancer Hospital in Brazilian remote rural areas, in comparison with conventional Pap test smears.

Methods: This analysis is a preliminary report from RODEO study that intends to evaluate the performance of LBC, manually screened, or screened by automation, presently running on Barretos Cancer Hospital, Clinics Hospital of Faculty of Medicine of University of São Paulo and Mendes de Barros Hospital. MU covers hundred of cities distributed along Brazilian remote rural areas. The rationale of this study was to evaluate the usefulness of SurePath LBC to improve cervical lesions diagnosis comparing with conventional Pap smear. The samples were randomly collected. All samples were blindly examined by cytotechnologist staff of Pathology Department. Only the manual screening arm was herein analysed.

Conclusions: We analysed data from 4402 women consecutively examined at MUs: 2898 (65.8%) examined by LBC and 1504 (34.2%) by conventional smears. No significant variation was found among the women when adjusted by age (t-test 0.276). The overall distribution of cytological examination have showed 4323 (98.2%) negative cases, 75 (1.7%) abnormalities and 4 (0.1%) unsatisfactory samples. Dichotomic analyses of LBC versus conventional smears have showed 2883 (97.8%) and 1490 (99.1%) normal/negative cases and 63 (2.2%) and 12 (0.8%) of abnormal cases, correspondingly (p=0.001). Specific cytologic alterations have also showed significant differences between the LBC and conventional smears (p=0.007), respectively: ASC-US27 (0.9%) and 3 (0.2%); ASC-H/AGC: 15 (0.5%) and 5 (0,3%); LSIL:15 (0.5%) and 2 (0,1%); HSIL: 6 (0.2%) and 2 (0.1%). No invasive cancer was detected in this series. This preliminary data have robustly support the superior performance of LBC to detect intraepithelial lesions and encourage us to consider the implementation of LBC in rural setting because despite of cytology diagnosis improvement, LBC residual material can be also used for molecular tests.

RAMAN AND FTIR SPECTROSCOPY, NEW TECHNOLOGIES FOR CERVICAL CANCER SCREENING AND HPV TESTING

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Objectives: Human papilloma virus (HPV) infection is a well established risk factor for cervical cancer. New technologies such as Raman and FTIR spectroscopy have recently been shown to accurately discriminate normal and abnormal cervical cytology samples based on the biochemical fingerprint of the cells. The aim of this study was to investigate the potential of Raman and FTIR spectroscopy to detect biochemical changes associated with HPV infection.

Methods: Four cervical cancer cell lines were investigated to determine if Raman and FTIR spectroscopy could discriminate between HPV positive and negative cells and between cells with different HPV type and copy number. Cervical cell lines, C33A (HPV negative), HeLa (HPV-18 positive, 20-50 copies per cell), SiHa (HPV-16 positive, 1-2 copies per cell) and CaSki (HPV-16 positive, 60-600 copies per cell) were cultured on glass slides. After Raman and FTIR measurements, the spectra were analysed using multivariate statistical techniques, Principal Component Analysis (PCA) and Partial Least Squares (PLS) analysis.

Conclusions: Each cervical cell line showed distinct spectral fingerprints corresponding to protein, nucleic acid and lipid levels. Principal Component Analysis (PCA) clearly differentiated the groups of spectra representing each cell line. Partial Least Squares (PLS) analysis was employed to construct a model which could predict the p16INK4A expression level based on the spectral fingerprint of each cell line. The results show clearly that as well as discriminating HPV positive and negative cells based on their biochemical fingerprint, cells with different HPV type and copy number could be clearly differentiated. In addition, the p16INK4A expression level could be predicted based on a spectral fingerprint of a cell. These results suggest that Raman and FTIR spectroscopy could potentially be used not only to detect abnormal cells in cervical cytology samples but also to detect the presence of HPV infection.
VALUE OF HPV-DNA TEST IN WOMEN WITH CYTOLOGICAL DIAGNOSIS OF ATYPICAL GLANDULAR CELLS (AGC) – A CROSS-SECTIONAL STUDY


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Objectives: Considering that endometrial abnormalities tend to be found in women with negative HPV test and squamous or glandular abnormalities of cervix usually are the positive HPV test, this study analyzed whether HPV test contributes towards defining histological abnormalities in women with atypical glandular cells (AGC) diagnosed at cervical cytology.

Methods: One hundred and eight women with conventional cervical cancer screening smears suggestive of AGC not otherwise specified (AGC-NOS) and favor neoplastic (AGC-FN) were consecutively enrolled. All women underwent colposcopic examinations and biopsy was performed according with the cytopathologic and/or colposcopic abnormalities present. All specimens were tested for high risk HPV genotypes by Roche’s polymerase chain reaction reverse line blot assay.

Conclusions: As results, final diagnosis revealed negative outcome in 80 cases (74%), cervical epithelial neoplasia 1 (CIN 1) in 13 cases (12%), CIN 2 or worse in 12 cases (11%) and glandular neoplasia in 3 (3%) cases. The overall detection rate of HPV was 21% (23/108). Neoplasia (CIN 2 or worse diagnostic) was significantly associated with positive HPV-DNA in women with AGC-NOS (OR=15.21; 95%CI: 2.64-87.50); however, there was no significant association between a histological diagnosis of neoplasia and HPV positivity in women with AGC-FN (OR=3.00; 95%CI: 0.36-24.92). The sensitivity, specificity, positive predictive value and negative predictive value of HPV-DNA testing for the detection of CIN 2 or worse in women with AGC-NOS were 71%, 86%, 29% and 97%, respectively. In women with AGC-FN, these values were 50%, 75%, 66% and 60%, respectively. In summary, HPV testing at the time of colposcopy for patients with AGC in whom no colposcopic abnormality is found may be a powerful ancillary tool for identifying women at a high risk of underlying significant cervical lesions.

L1 REGION SEQUENCING TO OVERCOME HPV GENOTYPING FAILURE BY A LINE PROBE ASSAY: ANALYSIS OF 856 ASCUS CERVICAL SMEARS FROM FRENCH WOMEN

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Introduction and objectives: Early detection of high-risk HPV types (HR) might help to identify women at high risk of cervical cancer. A genotyping of HPV positive samples is achieved by a variety of methods such as line probe assays. With these methods, a significant number of HPV remain untypable. In the present study, direct sequencing was used to characterize HPV that were not genotyped by a commercially available reverse hybridization assay.

Methods: During three years, 2007-2009, HPV detection was performed on a continuous series of 856 ASCUS cervical smears with Inno-Lipa HPV Genotyping Extra (Innogenetics). This assay can identify 18 HR HPV (16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 66, 68, 73, 82), 7 low-risk (LR) (6, 11, 40, 43, 44, 54, 70) and 3 genotype of indeterminate oncogenic risk (69, 71, 74). In case of genotyping failure, amplification of the L1 region was carried out using universal primers (MY09, MY11, HMB01) and the genotype was determined by comparison with sequences in the NCBI data base.

Results and conclusion: Among 856 cervical smears, 441 had detectable HPV DNA (51,5%) and 333 were positive with a HR HPV. Genotyping was a failure for 67 mono-infected samples. After sequencing, genotype was successfully obtained for 27 samples (40%): 5 were HR HPV (type 39, 52, 56, 58 and 67), 21 were LR HPV (type 55, 61, 62, 81, 83, 84) and one was associated with intermediate risk (type 67).

LR genotypes found after sequencing among untypable were not included in the typing assay and therefore can not be considered as failure. On the contrary, the line-probe assay failed to detect 5 HR HPV, that is 1,5% of the total number of HR HPV samples. Consequently, the presence of a highly oncogenic HPV can not be ruled out in case of genotyping failure and L1 sequencing should be performed.
HPV mRNA TESTING - LONG TERM FOLLOW UP OF 20,000 WOMEN IN NORWAY

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Objective: The objective of this study is to assess long term follow up of women with HPV mRNA test at the University Hospital of North Norway.

Methods: Our study comprises samples from approximately 20,000 women who had an HPV mRNA test in 2003 and 2004, originating from routine clinical practice of GPs and gynecologists in Norway. The HPV mRNA test PreTect HPV-Proofer detects and types E6/E7 mRNA from the five main high-risk genotypes 16, 18, 31, 33 and 45. Of 20,307 HPV mRNA tests, 1,890 (9.3 %) were positive: 5.1 % HPV 16, 1.5 % HPV 18, 0.5 % HPV 31, 2.0 % HPV 33 and 2.0 % HPV 45. 171 women (0.8 %) were positive for two HPV types and 21 women (0.1 %) were positive for three HPV types. HPV mRNA data will be correlated to follow-up data (cytology and histology) from the Norwegian Cancer registry up to December 2010 (at least six years of follow up).

Conclusion: Long term follow up of 20,000 women in Norway with an HPV mRNA test result in 2003/04 will be presented.

HPV MRNA AND IMMUNOCYTOCHEMISTRY FOR HIGH GRADE CERVICAL DISEASE DETECTION IN LIQUID-BASED CYTOLOGY SPECIMENS

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Objectives: Routine liquid-based cytology (LBC) provides an excellent method to detect high grade squamous intraepithelial lesions (HSIL) based upon atypical morphology. For HSIL cases, high specificity is off-set by low sensitivity. Conversely, using low grade lesions as the diagnostic threshold, sensitivity increases with a corresponding reduction in specificity. Ideally, combining morphology assessment with adjunctive tests possessing high positive predictive value for high grade cervical disease detection would create a useful paradigm of detecting HSIL lesions with both high sensitivity and high specificity, thereby reducing the number of unnecessary colposcopies. The current study evaluated the performance of LBC cytology with ProEx C (BD Diagnostics) immunocytochemistry (ICC) and the PreTect HPV-Proofer assay (NorChip) for the detection of biopsy confirmed high grade cervical disease (CIN2+).

Methods: Cervical cytology specimens collected into BD SurePath preservative fluid (BD Diagnostics) and categorized as ASC-US+ on cytology were included in the study. An additional LBC slide was prepared for ICC staining using ProEx C (BD Diagnostics) containing antibodies to MCM2 and TOP2A which detect aberrant S-phase induction. RNA was isolated from the residual enriched cell pellet using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion) and utilized in the PreTect HPV-Proofer assay designed to detect E6/E7 mRNA from HPV types 16, 18, 31, 33 and 45.

Conclusion: A total of 85 cervical cytology specimens were analyzed with biopsy results on 73 patients. Positivity for the ProEx C and HPV-Proofer assays by cytology category was 50% and 30% (ASC-US), 36.8% and 63.2% (LSIL), 100% and 100% (ASC-H) and 90.9% and 72.7% (HSIL), respectively. ProEx C was found to have a sensitivity of 84.8%, specificity of 66.7%, PPV of 81.3%, and NPV of 72% for detection of CIN2+. Among the subset of 73 patients for which both ProEx C and HPV-Proofer results were available, the sensitivity for each assay was 92.3% and 84.6%, specificity 60% and 50%, PPV 75% and 68.8%, and NPV 85.7% and 71.4%, respectively. Both ProEx C and the PreTect HPV-Proofer assay increase PPV for the detection of CIN2+ disease over cytology alone, with ProEx C appearing to have better clinical performance in this pilot study.
HPV-mRNA DETECTION IN THE FOLLOW-UP OF PATIENTS WITH HPV INFECTIONS


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Objectives. HPV DNA has been identified in almost all cervical cancers and women with active HPV infection express E6/E7 oncogenes. As only a small proportion of infections progress towards cancer, it is important to distinguish transient HPV infections from persistent or progressive ones.

Methods. 362 samples were tested by conventional pap smear; HPV-DNA test and typing (Innogenetics N.V. Belgium); E6/E7-mRNA expression from the carcinogenic HPV types 16, 18, 31, 33, 45 (PreTect HPV-Proofer assay, NorChip, Italy). Statistical tests were carried out using STATA 10.1 software. Data obtained were correlated through K-statistic value with the aim of identifying possible significant associations.

Conclusions. Pap-smear: negative (NEG) = 248 (68.5%); atypical cells of undetermined significance (ASCUS) = 50 (12.4%); low-grade squamous intraepithelial lesion (LSIL) = 36 (9.9%); high-grade squamous intraepithelial lesion (HSIL) = 28 (7.7%).

The HPV-DNA test was positive in 192/362 (53.04%) samples; the HPV-mRNA test was positive in 80/362 (22.10%) samples. In addition, the HPV-DNA test was positive in 110/248 (44.4%) of NEG, 37/50 (74.0%) of ASCUS, 22/36 (61.1%) of LSIL and 23/28 (82.1%) of HSIL; the E6/E7-mRNA test was positive in 25/248 (10.1%) of NEG, 17/50 (34.0%) of ASCUS, 15/36 (41.7%) of LSIL and 23/28 (82.1%) of HSIL.

The detection of HPV-mRNA shows a greater association with the degree of development of atypical or malignant lesions in comparison to the presence of HPV-DNA. Therefore the mRNA test might be a potential second level tool for the appropriate follow-up of ASCUS and LSIL patients with persistent or progressive HPV infections.

HPV E6/E7 mRNA AND HPV DNA TESTING IN POST COLPOSCOPIC TREATMENT SURVEILLANCE OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background: The risk of disease recurrence post treatment with LLETZ for high grade CIN ranges from 5-35%. Women with evidence of persistent HPV infection following treatment have a higher incidence of disease recurrence than those who clear their HPV post-treatment. The aim of this study is to evaluate the utility of both HPV DNA and mRNA testing in post treatment surveillance of cervical disease.

Study Design: To date, 1,020 women presenting at colposcopy at the Coombe Women and Infants University Hospital have been prospectively enrolled in the study. Cervical cytology specimens are taken at first visit prior to colposcopic procedure and at regular intervals (approximately 6, 12 and 18-24 months) during follow up. HPV DNA is detected using HC II assay (Qiagen) and HPV mRNA is detected using HPV PreTect Proofer (Norchip)

Results: 543/1020 women recruited, have been treated for cervical disease. The data presented in this abstract relates to 349 patients treated by LLETZ for low grade and high-grade disease. The prevalence of high risk HPV DNA and mRNA in these women prior to treatment was 93.2% and 76.6%, respectively. HPV 16 was the most predominant HPV type representing 69% of the cohort. Histological examination revealed 79% had CIN2+ disease, 15.6% CIN1, and 2.6 % Normal. Post colposcopic follow up of these women, at an interval of between 6-12 months post treatment, indicated HPV DNA persistence in up to 22% of cases, HPV mRNA persistence in 9.5 %, of which 20% had abnormal cytology.

Conclusions: HPV DNA/mRNA testing is useful for predicting recurrence of CIN in women treated for high grade CIN and can be used as test of cure in the colposcopy setting.

This study is funded under the CERVIVA Programme by the Health Research Board, Ireland.
EVIDENCE OF PGMY-PCR COMPETITION AND BIAS IN THE DETECTION OF HPV DNA IN AN ASC-US POPULATION

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Objectives: PCR methods directed at the L1 gene are commonly used to determine HPV type and have been reported to be challenged by samples with mixed infections. We report an informatics analysis suggesting PCR competition may also contribute to the rate of false-negatives when PCR-based methods are used to determine the presence of “high-risk” (HR) types of HPV. An informatics analysis predicts a strong bias against the A5 and A6 species groups. A study group consisting of ASC-US subjects was used to test this prediction.

Methods: The PGMY09/11 primers were analyzed for affinity to each of 42 types of HPV that are reported to be amplified. We considered the degree of pairing, the predicted Tm of each pairing, and the number of primers that could amplify each HPV type under standard thermocycler conditions. Types were ranked by overall affinity, with the highest ranked types having the greatest number of primer combinations that could be used to amplify the virus. Additionally 1,368 ASC-US ThinPrep® samples positive for HR HPV DNA by the CERVISTA® HPV HR test were tested using PGMY09/11 PCR and bidirectional sequencing and the results compared to the informatics predictions.

Conclusions: The results suggest that HR types in the A9 species group can be preferentially amplified over the A7, A6, and A5 species and predict that for samples typed to HPV species by PCR/sequencing and an independent method, the percent agreement would be lowest for the A5/A6 groups and highest for the A9 group. Percent positive agreements between PCR/sequencing and CERVISTA HR HPV was 94% for high-risk HPV, 82.9% for species group A9, 72.5% for A7 and 61.3% for A5/A6 in this analysis. PCR competition is an understudied factor in the determination of viral prevalence in populations. Informatics predicts a strong bias against the HPV A5 and A6 species groups and this was observed in this study of ASC-US cytology samples. The analysis of this study population with CERVISTA HPV HR and PCR/sequencing results reveals positive agreement by species group that is consistent with this prediction. More investigation is warranted to determine the impact of PCR-bias on HPV prevalence studies.

SENSITIVITY AND SPECIFICITY OF THE CERVISTA® HR HPV TEST AS COMPARED TO HYBRID CAPTURE® 2 FOR WOMEN 30 YEARS AND OLDER

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Objectives: Routine screening for high-risk HPV is currently being used, in conjunction with cytology, to triage patients for their risk of developing cervical cancer. In the U.S. the FDA has approved two methods for screening women for high-risk HPV. In this study, Cervista and Hybrid Capture 2 (hc2) were compared for sensitivity and specificity with regard to CIN 2 or greater histology (CIN2+) and to each other in a population of women 30 years and older (30+).

Methods: The data for this analysis, which included over 8,000 women, was provided by the SHENCCAST II study authors1. The relative specificity and sensitivity of Cervista was compared to hc2 using a non-inferiority analysis as described by Meijer et al. 20092. Clinical specificity and sensitivity was calculated by comparing molecular test results to histology.

Conclusions: The Cervista® and hc2 test results were compared for the 212 women 30 years of age or older with CIN2+ histology and for the 7,218 30+ women with ≤ CIN 1 histology. Among those with CIN2+, 203 were positive for HPV using hc2, 9 were negative, 197 were positive using Cervista and 15 were negative. The clinical sensitivities of hc2 and Cervista were 95.8% and 92.9% respectively (P=NS, not significant). Among the ≤ CIN 1 women, hc2 was positive in 809 cases and negative in 6,409. Cervista was positive in 637 and negative in 6,581 cases. The clinical specificities of the two tests are 88.8% and 91.2% for hc2 and Cervista respectively (P <0.0001). Meijer proposed a non-inferiority analysis for the comparison of a high-risk HPV test to a reference test for use in a clinical setting. This analysis uses a polynomial statistic to compare sensitivity and specificity to a relative sensitivity and specificity requirement. The proposed analysis requires a relative sensitivity of 90% and a specificity of 98% or better. Cervista was compared to hc2 as the reference. The Cervista test met the 90% relative sensitivity requirement with a probability of P > 0.0006. Cervista also met the 98% specificity requirement with a probability of P > 0.0001. The Cervista test meets the proposed non-inferiority criteria.

HUMAN PAPILLOMAVIRUS-TYPE SPECIFIC RISK FOR PROGRESSIO OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Objectives: To understand the association between human papillomavirus (HPV)-type and risk of progression we conducted a prospective cohort study to identify HPV-type-specific risk for progression of atypical squamous cells of undetermined significance (ASCUS) and CIN.

Methods: Subjects included 1671 Japanese women undergoing cytological screening, who had abnormal cytology (ASCUS, LSIL, HSIL) and underwent HPV genotyping. Women with cytological diagnosis of HSIL were subjected to colposcopy. Of these, 204 cases that were confirmed as ?CIN3 and 67 cases of multiple HPV infection were excluded from further analyses. The remaining 1400 cases included 68 ASCUS, 969 LSIL and 363 HSIL with histologically proven CIN2. The seven most prevalent oncogenic HPV genotypes in Japanese women (16, 18, 31, 33, 35, 52, 58) were classified as high-risk HPV and other oncogenic HPV types as unclassified HPV. Cumulative progression risk to $\geq$CIN3 was investigated. During follow-up, 174 cases progressed to $\geq$CIN3. Five-year cumulative progression rate and time-to-progression from LSIL and CIN2 to $\geq$CIN3 were strongly related to HPV status. Among high-risk- HPV types, cumulative progression risk varied. Cases with HPV(16/18/33) had a higher progression rate than HPV(31/35/52/58) for LSIL and CIN2 (p=0.0002 and p=0.002, respectively). Furthermore, HPV (16/18/33) had a shorter time to progression than HPV (31/35/52/58) for CIN2 (p=0.003).

Conclusions: Our findings suggest that progression of CIN in terms of cumulative rate and time to progression is related to HPV genotype. HPV 16, 18, and 33 have a significantly higher risk of progression than other high-risk HPV types. HPV genotyping is useful for individualized management of LSIL and CIN2.

DETECTION OF ONCOGENIC HUMAN PAPILLOMAVIRUS (HPV) IN ANAL SPECIMENS USING HYBRID CAPTURE 2: COMPARING COLLECTION IN THINPREP OR STM MEDIA

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Objectives: Current screening for anal cancer includes cytology followed by high-resolution anoscopy (HRA). Oncogenic HPV infection causes most anal and cervical cancer as well as the precursor lesion of high-grade dysplasia (AIN2+). In cervix, oncogenic HPV is detected by the FDA-approved Hybrid Capture 2 assay (HC2, Qiagen). Samples for the HC2 assay could be residual liquid-based cytology (LBC) specimens or co-collected specimens in Sample Transport Medium (STM, Qiagen). This study compared HC2 test performance using either residual anal cytology specimens in ThinPrep medium (Hologic) or co-collected STM anal specimens. Performance of HC2 and liquid-based cytology as a predictor of AIN2+ was also compared. This study was conducted for research purposes only.

Methods: A prospective study was performed on 298 patients referred for anal cancer screening. All patients had two specimens sequentially co-collected: first, a swab into ThinPrep medium for LBC with a Gyn or Non-Gyn filter followed with residual tested by HC2. Then, a randomized swab or brush into STM for another HC2 assay. HRA was performed on all patients. HC2 was performed on all specimens. Biopsies were taken from all lesions suspicious for AIN2+. A PCR for b-globin determined the amount of cells in LBC specimens.

Results: The sensitivity of HC2 against a disease end-point (AIN2+) for the STM specimens (91%) was significantly higher (p=0.0053) than cytology (77%). The swab or brush used for STM specimens provided similar clinical sensitivity, but assay signal was higher for brushes. The clinical sensitivity of HC2 for residual LBC specimens was lower than for the STM specimens. The sensitivity for LBC was 85% using a GYN-filter, but was 62% using the Non-Gyn filter. For LBC specimens that were AIN2+, the b-globin amount was significantly lower (p=0.0011) for HC2 negative specimens compared with HC2 positives.

Conclusions: Clinical performance of the HC2 assay was best for STM specimens. For residual LBC specimens, HC2 was best when specimens are processed with the GYN filter compared to the NON-GYN filter. The apparent false negative results for LBC specimens were probably due to low residual material after cells for cytology were taken. The HC2 assay provides higher sensitivity than cytology for diagnosis of AIN2+.
COMPARISON OF THE CERVISTATM HPV ASSAYS AND HPV 4 ACE FOR THE DETECTION OF HPV 16/18 IN HYBRID CAPTURE 2 POSITIVE MEDIA

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Objectives: To validate efficacy of the novel human papillomavirus (HPV) genotyping method, the CervistaTM HPV high risk (HR) and HPV 16/18 tests

Methods: Besides liquid-based cytology, we collected additional cervical swab samples, 197 cases, for HPV genotyping by a PCR method, HPV 4 auto-capillary electrophoresis (ACE), and CervistaTM HPV HR and HPV 16/18. To compare the clinical accuracy of the HPV 4 ACE test and the CervistaTM HPV HR test to detect lesions higher than LSIL, we also calculated the sensitivity and specificity compared by the paired proportion test. Agreement between the HPV assays was assessed by Cohen’s kappa statistic.

Conclusions: The CervistaTM HPV HR test and PCR method or the HPV 4 ACE test showed substantial agreement for detection of HR HPVs (81.7%, kappa=0.767 / 87.3%, kappa=0.744, p-value<0.001). And the CervistaTM HPV 16/18 test also showed substantial agreement with the HPV 4 ACE in the detection of HPV 16 and HPV 18 genotypes (89.5%, kappa=0.628, p-value<0.001). Also, in correlation with cytologic results, the sensitivities and specificities of the CervistaTM HPV HR test and the HPV 4 ACE were 84.5% vs. 91.4% and 72.7% vs. 73.4%, respectively, when those higher than low-grade squamous intraepithelial lesions were regarded as abnormal cytology. Increased viral load of HC2 raised the predictive value of the final pathology, but it was difficult to find to the exact cut-off value to expect cervical intraepithelial neoplasia (CINs). On the other hand, HPV genotyping tests for HPV 16/18 predicted CINs better than HPV DNA tests for HR HPV (28.6-66.7% vs. 21.5-25.9%). HPV genotyping tests for HPV 16/18 must be considered to predict more CINs. The CervistaTM HPV HR test was approved by FDA for the HR HPV detection and the CervistaTM HPV 16/18 test for HPV 16/18 genotyping in 2009. And the CervistaTM HPV HR and the CervistaTM HPV 16/18 tests are valuable tools for the detection of high-risk HPVs and for genotyping of HPV 16 and HPV 18-4.

EUROPEAN HPV DNA TEST EXTERNAL QUALITY ASSURANCE SCHEME (EHEQAS)

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Objective: HPV DNA testing has been recommended by Guidelines of American and European organizations as a method for (a) triage of women with atypical cytology, (b) follow up of women treated for precancerous cervical lesions, (c) detection of women at risk of cervical cancer. In order to assess and improve the quality of performance of expert laboratories, we have set up EHEQAS.

Methods: Any European laboratory performing HPV tests may participate (low or high-throughput, low or high-resolution typing, in-house or commercial test). Batches of 5-13 samples are sent from the coordinator (Dr Neophytou) to participants 2-3 times per year. Participants test samples and have to report results to the coordinator within 3 weeks. Results are tabulated and consensus results are prepared. Marks are awarded to participants and once every 12-18 months certificates (of participation or competence) are issued. Competence certificates are based on the average marks received in ≥3 rounds of tests.

Results: EHEQAS began its operations in 2006 with 5 participating laboratories. In 2010 already 15 laboratories have participated from 6 European countries. So far 127 samples have been tested in 11 rounds. Consensus results show 38/127 negative samples, 40/127 had single infections and 49/127 had co-infections. More than thirty genital types have been detected in the positive samples. Comparison of results showed the preferential amplification of different types by different primer systems: e.g. MY09/11 amplifies better types 52 and 53 whereas GP5/6+ system amplifies better types 31 and 42. Six laboratories have been issued with a certificate of competence in September 2009 and eight laboratories will receive such a certificate in February 2011.

Conclusion: Participation in EHEQAS improves the quality of expert laboratories performing HPV DNA testing and provides a platform for (a) improvement and development of HPV diagnostic tests, (b) compliance with ISO 17043 and ISO 15189.
SELF-SAMPLING FOR CAREHPV DNA TESTING: EXPERIENCE FROM THREE CONTINENTS
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Objectives: Cervical Cancer is still a leading cause of cancer-related deaths in women, with most of the burden occurring in developing countries. It is imperative that implementation of new screening strategies increase coverage and are high-quality, affordable, and culturally acceptable. PATH is collaborating with public-sector institutions from Nicaragua, Uganda, and India in demonstration studies to evaluate the acceptability, feasibility, and performance of the careHPV™ Test.

Methods: After completion of the consenting process, women receive simple guidance on how to self-collect a vaginal sample, which is done in a private setting and used for careHPV testing. A health worker then performs a pelvic evaluation and collects two direct samples from the cervix for careHPV testing and conventional cytology. Finally, VIA with 5% acetic acid is done. Any woman with any positive test goes to colposcopy and biopsy if needed. Histological diagnosis of CIN2+ was used as the gold standard for evaluating screening tests.

Over 7,000 women have been enrolled in the study; the overall acceptability of self-collection vaginal sampling was 83%, ranging from 75% in Andhra Pradesh and 78% in Nicaragua to greater than 98% in Uganda and Uttar Pradesh. CareHPV testing using self-collected vaginal samples was highly sensitive in Nicaragua (82.1%, 95% CI: 66.5–92.5) and Hyderabad (75.6%, 95% CI: 59.7–87.6) for detecting CIN2+ lesions. These results are very comparable to careHPV using cervical samples collected by a healthcare provider (84.6% in Nicaragua; 85.4% in Hyderabad), and higher than the overall sensitivity obtained by VIA (69.8%, 95% CI: 58.9–79.2) or Pap smear (58.1%, 95% CI: 47.0–68.7).

Conclusions: Self-collection of vaginal samples for cervical cancer screening is very well accepted by women from India, Nicaragua, and Uganda, and was highly sensitive for detection of precancer and cancer. New strategies incorporating self-collection of vaginal samples at the community level would allow us to achieve high coverage of women at risk as well as to make better use of scarce resources available in those settings, since pelvic evaluation would be needed only for those with positive HPV results.

COMPARISON OF A NEW AUTOMATED MULTIPLEX REALTIME HIGH RISK HPV ASSAY WITH HYBRID CAPTURE 2 IN A LARGE GERMAN ROUTINE LABORATORY

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Objectives: Evaluation of the new automated Abbott RealTime High Risk HPV assay (RealTime-HPV) in comparison to Digene Hybrid Capture 2 (hc2, Qiagen) with cervical specimens from a large German routine laboratory.

Methods: 505 liquid cytology specimens (PreservCyt, Hologic) referred for cytology and high risk (HR) HPV DNA testing (hc2) were run with RealTime-HPV on the m2000 System (Abbott). This multiplex real-time PCR assay detects 14 HR HPV types and simultaneously differentiates between HPV 16, HPV 18 and 12 non-HPV 16/18 HR types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in a single test, while hc2 targets the same HR types, except HPV 66. RealTime-HPV amplifies human ß-globin in the same reaction to ensure appropriate sample cellularity and to control DNA extraction efficiency and potential PCR inhibition, whereas hc2 does not contain an internal control. Samples with discordant results between both HR HPV assays were further analyzed with Roche Linear Array, targeting 37 HPV genotypes. RealTime-HPV and hc2 results were correlated with histology data (routine pathology review) available for 280: 47 <CIN2 (17%), 71 CIN2 (25%) and 162 CIN3+ (58%).

Results: Overall agreement between RealTime-HPV and hc2 on 505 specimens was 85.5%; analytical sensitivity, specificity and accuracy of RealTime-HPV (96.9%, 96.3%, 96.6%) were significantly higher than with hc2 (92.3%, 81.3%, 88.9%). HR-HPV detection rates in <CIN2 cases were lower with RealTime-HPV (80.9%) than with hc2 (89.4%). Similar HR HPV detection rates in histologically confirmed high grade lesions were observed with RealTime HPV (CIN2+ 90.6%; CIN3+ 90.7%) and hc2 (CIN2+ 89.3%; CIN3+ 88.3%); numerical differences between both tests were statistically insignificant. A high correlation of HPV 16/18 detection rates with increasing histological severity was observed with RealTime-HPV, while detection rates of other HR HPV decreased with severity of CIN.

Conclusions: Clinical sensitivity of RealTime-HPV was comparable to that of hc2, while analytical performance of RealTime-HPV was superior to hc2. Discrimination of HPV 16/18 from other HR HPV types provides additional information for risk stratification in patient management.
ANALYTICAL TESTING OF NEW HPV GENOTYPING TECHNOLOGIES, digene© LQ, RH AND PS SYSTEMS.

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Objectives: To evaluate three different systems, digene® HPV Genotyping RH, LQ and PS Test, for HR-HPV detection and typing in clinical samples with and without cervical abnormalities. Baseline data are available using Hybrid Capture 2 High Risk HPV (HC2, Qiagen) detection and Linear Array (L.A., Roche Diagnostic) genotyping.

Methods: A total of 305 STM samples were analyzed using of HC2 technology for HPV detection in the context of the preventive strategies of Catalonia, Spain. For PS Test (HC2-based genotyping test for the detection of HPV16, 18 and 45), 750 ìl of each denaturalized STM sample was used directly. LQ and RH are Gp5+/6+ PCR-based tests for the detection of 18 different HPV genotypes. LQ Test is a bead-based xMAP technology for the detection of HPV by fluorescence using LiquiChip; RH Test is a stripe–based genotyping system for HPV detection by colorimetric methods. DNA from the samples was extracted using the QIAamp® MinElute® Virus Spin Kit and amplified according to the manufacturer’s instructions. Concordance, Kappa index (k) and McNemar’s tests were calculated for the comparisons of each test with the reference values, HR-HC2 and LA results.

Conclusions: The overall concordance in HPV detection was 98.36% (k=0.924) when comparing the four techniques. The concordances and kappa statistics for each technique compared with HC2 were 98.03% (k=0.91) for RH, 98.69% (k=0.94) for LQ and 91.80% (k=0.82) for PS. There was a very good agreement in HPV type specific concordance for the most prevalent types HPV16 (kappa range=0.83-0.90), 18 (k.r.=0.74-0.80) and 45 (k.r.=0.82-0.90). No cross reactivity with low risk types was identified.

In conclusion, LQ, RH and PS tests show good validity when compared with the FDA approved HR-HC2 system and with LA technique.

VERIFICATION OF THE QIASYMPHONY DSP AXpH DNA KIT AS FULLY AUTOMATED SAMPLE PREPARATION FOR PRESERVCYT® SPECIMENS

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Objectives: We have developed and verified a DNA extraction procedure based on pH-driven anion exchange sample preparation chemistry (AXpH) for use on the QIAsymphony SP. The fully automated procedure allows processing of 4 ml liquid based cytology media. This work summarizes data generated in the verification process using the HC2 High-Risk HPV DNA Test® (HC2 test) for downstream analysis.

The QIAsymphony applications presented here are for research purposes. Not for use in diagnostic procedures.

Methods and Results: To compare manual and automated sample preparation methods, DNA was isolated using the QIAsymphony DSP AXpH DNA protocol and the eluates were tested with HC2. Testing was conducted using either pools of de-identified, clinical PreservCyt samples or low positive cell culture samples. We evaluated the performance characteristics of the automated DNA extraction procedure for repeatability, precision, sample stability on the worktable and eluate stability. Repeatability: DNA was purified from 7 dilutions of a HPV positive cell line in two independent experiments using the QIAsymphony DSP AXpH DNA Kit. The correlation coefficient RÇ was 0.991. Precision: Inter-instrument and inter-day precision was determined on three different QIAsymphony instruments and on three different days using a HPV positive cell line. Inter-instrument and inter-day precision was 22.3% and 20.5%, respectively. Sample stability on the worktable: Pools of positive and negative clinical pools or positive cell culture material were stored for 0, 4, 8 and 12 hours on the QIAsymphony worktable prior to DNA extraction. Storage of the samples did not have a significant impact on the observed RLU/co when tested with HC2. Eluate stability: DNA extracted from positive and negative clinical pools or positive cell culture material were stored for up to two weeks at 4-8°C or up to one month at –20°C prior to analysis in HC2. Storage at 4-8°C or –20°C did not lead to a reduction in observed RLU/co signal.

Conclusion: We successfully evaluated the performance characteristics of the automated QIAsymphony DSP AXpH DNA extraction procedure using PreservCyt samples in combination with the HC2 High-Risk HPV DNA Test®.
**THE COBAS®4800 HPV TEST FOR HIGH RISK HPV DNA DETECTION**

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**Objectives:** High risk (HR) HPV DNA testing is highly sensitive to identify women with or at risk of CIN2+ lesions. Therefore, it represents an attractive alternative to the Pap smear for cervical cancer screening. We compared the results obtained with the new cobas®4800 HPV Test to those obtained with the HC2 test, analyzed discordant results by specific genotyping and determined if Ct values given by the cobas®4800 can reflect viral load.

**Methods:** 322 cervical samples (88% with ASC-US cytodiagnosis) stored in PreservCyt were routinely tested for the presence of HR HPV DNA by HC2. An aliquot was also tested with the cobas®4800 according to the manufacturer’s instructions. Samples with discrepant HR HPV results were tested with the INNO-LiPA HPV extra. In addition, all specimens were analyzed using a homemade real time PCR allowing HPV16 and HPV18 DNA quantification.

**Conclusions:** HR HPV prevalence was similar by cobas®4800 (45.3%) and HC2 (47.2%) and agreement between the two assays was 86% (kappa 0.71). Among the 24 cobas- but HC2+ samples tested with the LiPA assay, 2 were LiPA negative, 16 harbored HPV types not targeted by the cobas®4800 and 6 were false negative. As for the 19 cobas+ but HC2- samples genotyped, 4 were LiPA negative, 6 harbored HPV DNA not detectable by HC2 (one HPV not targeted by HC2 and 5 HPV16 with a viral load under the HC2 limit of detection) and 9 were true positive. When taking into account the true discordant results confirmed by the LiPA, agreement between the two assays reached 93% (kappa 0.86). The log transformed values of HC2 plotted against the cobas Ct values showed a linear relationship between both parameters. Moreover, HPV16 and HPV18 DNA were detected as efficiently by the cobas®4800 and our home made quantitative real-time PCR (agreement of 98%) with Ct values highly correlated. This suggests that the cobas®4800 could be used as a quantitative test.

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**QIAGEN LIQUICHIP AND LINEAR ARRAY ASSAYS IN A MULTICENTER STUDY OF A POPULATION OF ASCUS PLUS AND HC2 POSITIVE PATIENTS REFERRED FOR COLPOSCOPY: THE 3M STUDY (MILAN, MARSEILLES, AND MADRID)**

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**Objectives:** New HPV genotyping assays are developed in order to specify the distribution of HPV types in several HPV population at risk for HPV infection. The concordance levels between each of the genotyping tests need be investigated. The primary aim of the study was to compare the clinical performance of two commercially available typing assays (Digene LQ and Roche LA) using a clinical cut-off of CIN2+ patients. The secondary aim was to comparatively assess the distribution of HPV types using these two assays.

**Patients and Methods:** The study population comprised 311 ASCUS+ women, HC II positive, who were admitted in three European referral gynecology clinics between 2007 and 2010: 158 patients from Madrid (Spain), 58 from Marseille (France), and 95 from Milan (Italy). A colposcopy with histological examination was performed for all these patients. The Digene LQ test utilizes probes for 18 HR HPV types (i.e., HPV 16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) was performed in the Luminex 100 IS System (Luminex Corporation). The LA detects 37 HPV genotypes by reverse line blot hybridization. A third in house RT-PCR HPV typing was performed in discordant digene/LA cases.

**Results:** The respective distributions of the HPV types using the Digene LQ® and LA were respectively HPV 16 (32%-21%), HPV 18 (4%-3%), HPV 31 (13%-8%), HPV 33 (5%-4%), HPV 35 (1%-2%), HPV 39 (2% for both tests), HPV 45 (3%-2%), HPV 51 (3%-4%), HPV 52 (3% for both tests), HPV 53 (2%-6%), HPV 56 (5%-4%), HPV 58 (4% for both tests), HPV 59 (1%-3%), HPV 66 (4%-3%), HPV 68 (2%-1%), HPV 73 (1%-2%), multiples HPV infection (14%-27%), and negative results (8%-1%). The sensitivity of the two assays for HPV typing was 92% for Digene LQ, and 99% for LA (considering that all patients are HCII HPV positive). The overall concordance between Digene LQ and LA was 93%; HPV genotyping of the discordant cases indicated that among the 21 cases LQ NEG / LA POS 12 were HPV negative using the homemade RT-PCR test, and nine were HPV positive using homemade RT-PCR. The absolute risk of being CIN2+ when having HPV16 is similar for both LA and QIAGEN: 60% and 61% respectively (23% and 25% of being CIN3+ when HPV16). The absolute risk of being CIN2+ when having HPV31 is also similar for both: 50% and 54% respectively (13% and 16% of being CIN3+ when HPV31). The relative risk of being CIN2+ when having HPV16+/HPV18- is 60% and 61% for Digene LQ and LA respectively (the risk of CIN3+ is 23% and 25% respectively). Data showed no significant difference in relative risk between tests.

**Conclusions:** These assays have a good clinical sensitivity to detect HPV types in CIN2+ patients, and allow in the same experiment to detect and determine the type of the virus. Our study showed no significant difference between Digene LQ and Roche LA for CIN2+, or CIN3+ diagnosis, and showed similar distributions of HPV types.
CLINICAL VALIDATION OF COBAS 4800 HPV TEST IN CERVICAL CANCER SCREENING IN CATALONIA

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Background: HPV testing with HC2 is applied in 3 settings in the Catalan screening population: 1- women with a cytological diagnosis of ASC-US; 2- women 40 years or older with a history of inadequate screening (no cytology in the past 5 years); 3- in the follow up after surgical treatment for HSIL. Validation of other tests should consider HC2 as the gold standard, according to the guidelines proposed by Meijer et al. 2009

Objective: To validate the Roche system Cobas 4800 for the detection of HPV in the cervical cancer screening population.

Methods: Sixty samples with a biopsy confirming ≥CIN2 were selected and used for the sensitivity study. For the evaluation of specificity, 898 samples without a diagnosis of ≥CIN2 were randomly selected from the screening population. Among these, there were 42 LSIL (27 with biopsy), 26 ASC-US and 830 negative (12 with biopsy and 818 without). The intra-laboratory reproducibility was evaluated in 546 samples, 32% of which were HC2 positive. Samples were collected in PreservCyt and cytology was done with the assistance of the Imager system (Hologic). HC2 and Cobas 4800 HPV tests were performed following the manufacturer’s recommendations.

Results: The Cobas 4800 HPV test showed a sensitivity for ≥CIN2 of 98.3% (95% CI:95.1-100) and a specificity for ≤CIN2 of 86.2% (95% CI:83.9-88.4). Sensitivity and specificity of HC2 were 98.3% (95% CI: 95.1-100) and 85.3% (95% CI:83-87.6) respectively. HPV 16 or 18, single or in a multiple infection, were present in 56% of HSIL cases.

Clinical sensitivity and specificity of Cobas 4800 were compared with HC2 by non-inferiority score test (relative sensitivity for ≥CIN2 of at least 90% and relative specificity ≥98% of the HC2). Both were non-inferior to these thresholds with p-value 0.0093 and p-value 0.0012, respectively.

A high reproducibility was observed in the intralaboratory study (kappa = 0.957).

Conclusions: These results show a good performance of Cobas 4800 as compared to the gold standard. Therefore, Cobas 4800 can be implemented in screening for cervical cancer.

OPTIMISATION OF A REAL-TIME PCR FOR HPV DNA DETECTION & LIPA GENOTYPING USING SPF10 PRIMERS

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Objectives: The SPF10 primers target a highly conserved 65bp region of the HPV L1 gene, which enables the amplification of numerous genital HPV genotypes. These primers make part of the INNO-LiPA HPV Genotyping Extra Amp kit (Innogenetics). After conventional PCR, the HPV amplicons are tested on the INNO-LiPA HPV Genotyping Extra assay to determine the exact genotype. As real-time PCR allows simultaneous amplification and detection, a real-time PCR approach with SPF10 primers would substantially diminish the workload and processing time by eliminating the LiPA step for the HPV-negative samples. In collaboration with Innogenetics, the application of SPF10 primers in a real-time PCR setting has been evaluated.

Methods: An in-house SYBR green real-time PCR protocol with SPF10 primers was developed on the LightCycler® 480 (Roche Applied Science) using HPV L1 plasmids (HPV6, 11, 13, 16, 18, 18b, 26, 30, 31, 33, 34, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 67, 68, 69, 70, 73, 74, 82).

Conclusions: HPV L1 plasmids for all genotypes could be detected with the SPF10 primers in a real-time SYBR green PCR. Ten-fold dilution series of HPV6, 16, 18 and 31 L1 plasmids (29700 to 29.7 copies) proved that a concentration of 29.7 copies could be picked up. Melting curve analysis showed a specific product with a melting temperature of approximately 81°C. The compatibility of the real-time PCR amplicon with the INNO-LiPA HPV Genotyping Extra assay will be evaluated. Moreover, the analytical performance of the in-house SYBR Green real-time will be further assessed using well-defined clinical samples.
COMPARISON OF THE PAPILLOCHECK® ASSAY WITH THE DIGENE HC2 HPV DNA ASSAY FOR THE DETECTION OF 13 HR-HPV IN CERVICAL AND ANAL SPECIMENS

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Objectives: In the present study, the PapilloCheck® assay was compared with the digene HC2 HPV DNA assay for the detection of 13 HR-HPV in 181 cervical specimens and 59 anal specimens.

Materials and methods: A cross-sectional survey of pregnant women followed in gynecology in Montpellier University Hospital Arnaud de Villeneuve and a cohort of HSH followed in medical care unit of infection diseases. Cervical samples were collected by means of brush and were preserved in a PreservCyt transport solution. Thin layer cytological smears were prepared by controlled membrane transfer technology using the ThinPrep 2000 processor (Cytic). The 2001 Bethesda classification was used for slide interpretation.

The Digene HC2 HPV DNA assay is based on a chemiluminescent reaction in which HPV DNA binds to an RNA cocktail probe to detect 13 HR-HPV types. The PapilloCheck® test (Greiner Bio-one, Frickenhausen, Germany) is a PCR-based DNA microarray system that allows to identify 24 HPV types.

Results and conclusions: Overall, 75 (30.5 %) samples were positive with the digene HC2 HPV DNA assay: 34 (18.8%) cervical samples and 41 (69.5%) anal samples. By considering only the 13 HR-HPV types detected by the digene HC2 HPV DNA assay, 66 (27.5%) samples were positive by the PapilloCheck® assay: 27 (14.9 %) cervical samples and 39 (66.1 %) anal samples. By considering all the HPV types detectable by the PapilloCheck® assay, the overall prevalence of HPV was 34.2% (82/240): 21.0% (38/181) for cervical samples and 74.6 % (44/59) for anal samples. Concordant results between the two assays were obtained for 225 (93.8%) samples with a Kappa coefficient value of 0.85, indicating an excellent agreement. Among the samples found positive with the PapilloCheck® assay, a multiple HPV infection (2 to 9 HPV types) was identified in 43 of 82 (52.4%) samples. The prevalence of HR-HPV, as determined by the PapilloCheck® assay, was 17.6% (36/205) in samples with normal cytology, 83.9% (26/31) in samples with LSIL or ASCUS, and 100% (4/4) in samples with HSIL. The PapilloCheck® assay may be considered as a reliable screening test for the detection and typing of HPV.

EFFECT OF DIFFERENT EXTRACTION METHODS AND A URINE CONSERVATION MEDIUM ON THE DETECTION AND RECOVERY OF HPV AND HUMAN DNA IN URINE

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Objective: Assess the impact of different extraction methods and an in-house Urine Conservation Medium (UCM) on detection and recovery of human papillomavirus and human DNA from urine.

Methods: First voided urine was provided by women with a cytological normal but HPV DNA positive cervical sample. One fraction of the urine was diluted, immediately after collection, with the UCM, one fraction remained untreated. Both fractions were stored for 3 days at room temperature and were subsequently frozen at -20°C until further processing. 200µl of each fraction was extracted with a Qiagen QiAamp DNA mini kit (QI) and 1ml was extracted with a Nuclisense EasyMag (EM) automate. Additionally two different concentration methods were applied. 1ml was subjected to centrifugation for 1h at 23000g and 4ml was concentrated with an Amicon Ultra filtration (AM) device. The DNA from the AM residue, the pellet (PP) and 200 µl of the supernatant (SN) obtained after centrifugation were also extracted using a Qiagen extraction kit. In total 14 donations from 11 different women were analysed. For each of the 140 DNA extractions the HPV DNA and the GAPDH gene were quantified by a real-time PCR.

Results: The number of copies of HPV and human DNA detected in 5µl of the eluent varied from zero to around and over 100.000 copies. For HPV DNA the best results were obtained by AM concentration, 8 of 14 samples without UCM and 12 of 14 samples with UCM were positive. Inferior results were obtained when analysing the SN, only 3 of 14 were positive for HPV without UCM and 7 out of 14 with UCM. For the GAPDH gene detection in the untreated urine, only AM and PP extractions were positive for all donations, 12 of 14 EM and QI extracts had also detectable human DNA. In the urine fractions diluted with UCM all donations scored positive for human DNA, including the supernatant. The median increase in detected copies by using the UCM was for AM, PP, QI, EM and SN respectively 46, 7, 6, 10, and 5 times.

Conclusion: The extraction method and the UCM have a major impact on the recovery of HPV and human DNA from urine.
VAGINAL SELF SAMPLING: PREFERENCES OF WOMEN.

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Objective: Vaginal self-sampling forms the ideal sampling method for population based HPV detection within a national screening program for cervical cancer prevention. The development of vaginal self samples has been investigator driven, without taking women's opinions into consideration. This study therefore investigates women's preferences for vaginal self-sampling.

Methods: Qualitative research methods were used consisting of structured interviews of 12 women regarding two different self sampling devices; vaginal lavage with the Delphi screener® (Delphi Bioscience BV Scherpenzeel, the Netherlands), and the Viba brush® (Rovers Medical devices, Oss, the Netherlands) with FTA cartridge® (Whatman, UK). Of these women, 6 were regularly screened (responders), and 6 were refused screening thus far (non-responders).

Conclusions: All women, both responders and non-responders to screening indicated that they would participate in screening if self sampling was introduced. The ideal self sampling device should be packed in a small box, sent by regular mail, easy to open, contain clear instructions (in cartoon form), and indicate referral to a website/telephone number for further information. The device itself has to be thin (< 1 cm) with a soft round tip, has a clear indication when it is inserted far enough, with maximal one additional instruction to take the sample. After sampling the device has to be put in a box or envelop, without further handling, in order to be sent by regular mail for further analysis.

In conclusion, women are very motivated to partake in screening using self sampling. The sample itself has to be women friendly, with clear instructions and minimal handling. On the basis of this study, new devices have been developed and will be shown during the congress.

EVALUATION OF A NEW SELF-SAMPLING DEVICE FOR HPV-TESTING

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Objective: The aim of this validation study was to evaluate a new self-sampling device for HPV-testing.

Methods: 120 women with an abnormal cervical smear were invited at our colposcopy to this study. By a written instruction the women used a new self-sampling device to collect a sample from the vaginae before they met the gynecologist who took the golden standard Liquid Based Cytology (LBC) test from the cervix. The self-sample device and an aliquot of the LBC were sent to the Microbiology Department in Malmö and analyzed for presence of HPV types by PCR and Luminex. The results from the two sampling methods were then compared. Only samples with high risk or potentially high risk HPV types were classified as HPV positive.

In 108 women both tests were able to be analyzed. HPV-positive tests were found in either the self-sampling or the LBC tests in 73/108 (68%). HPV-positivity in the self-collected samples were detected in 65/108 (60%) and in the LBC 64/108 (59 %). The sensitivity for the vaginal self-sampling-test was 89% (95% CI 0.80-0.95). 9 vaginal samples 9/108 (8%) showed HPV-positivity whereas the corresponding LBC samples where HPV negative.

Conclusions The new self-sampling device for HPV-infection seems to be an effective way to screen patients for high or potentially high risk HPV-infections. Since this new self-sampling device is cheap and easy to use, it has a great potential to reach women who do not attend the screening-program.
ANALYTICAL AND FUNCTIONAL ANALYSES OF AN AUTOMATED PAN-HPV ISH ASSAY FOR CERVICAL BIOPSY TISSUE

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Objectives: The histopathologic interpretation of cervical intraepithelial neoplasia (CIN) is important for patient management. Various studies document significant rates of discordant diagnostic H&E interpretation results among pathologists. Specifically, a substantial number of false-positive diagnoses have been made on cases negative for dysplasia (no-CIN). These cases may closely mimic CIN at the morphologic level. We designed a human papillomavirus (HPV) in situ hybridization (ISH) probe* that includes the most common high and low risk HPVs known to cause CIN lesions. The HPV ISH probe is used within an assay to detect the presence of the virus within formalin fixed, paraffin embedded (FFPE) cervical biopsy tissue. The absence of HPV staining in cervical biopsies may support a no-CIN histological interpretation and aid pathologists in minimizing over-diagnosis. This study describes the analytical and functional criteria of this novel assay.

Methods: The digoxigenin-labeled HPV probe binds high and low risk HPV viral types and is visualized by blue chomogenic alkaline phosphatase detection system. The stain appears as a nuclear-specific blue pigment deposit within HPV infected cells of FFPE cervical biopsy specimens. The assay was fully automated on the Ventana BenchMark series of instruments. Analytical sensitivity and specificity, detection limit, interference, and reproducibility studies were conducted.

Conclusions: The HPV ISH assay* demonstrated staining within xenografts– CaSki (400-600 copies of HPV16), HeLa (10-50 copies of HPV18), and SiHa (1-2 copies of HPV16). No HPV staining was detected within C33 xenograft (0 copy of HPV virus). Further, this assay demonstrated no cross-hybridization to Epstein-Barr virus (EBV), cytomegalovirus (CMV) or herpes simplex virus (HSV) 1 and 2. The probe proved no sequence-dependent cross-reactivity on normal human chromosomal metaphase spreads. The agreement rates for inter-instrument, inter-run (day to day), and inter-observer comparison were all 100%. This assay may be useful as an adjunctive test to H&E and may improve the accuracy in differentiating CIN versus no-CIN lesions within cervical biopsy specimens.

* Disclaimer: This is not a commercially available product.

HPV GENOTYPING ON SUREPATH™ PRESERVATIVE USED FOR PRE-FIXATION OF CONE BIOPSIES.

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Objectives: The National guidelines in Denmark, as well as in many other countries, recommend HPV-DNA testing on ASCUS cases and on the first PAP-smear after conisation. HPV finding after a cone, which can be a re-infection or a persisting infection, may indicate residual lesion or a risk for relapse. This risk is probably higher if there is a persisting infection, i.e. the post-cone HPV is of the same genotype as the pre-cone one. Conisation is mostly performed on high-grade lesions, where the HPV testing is usually not performed. HPV testing on paraffin-embedded tissue from cone biopsies is a time consuming and demanding process. The aim of this pilot study was to test the feasibility of HPV genotyping on SurePath™ preservative fluid that was used for pre-fixation of the cones.

Methods: 24 cone biopsies were received in SurePath™ vials that are primarily designated for liquid-based PAP-smears. A SurePathTM PAP-smear was also sampled just before the conisation in all cases. After a pre-fixation in SurePath™ vials for at least 6 hours, the cones were re-fixed in formalin and processed accordingly to the laboratory’s procedures for formalin fixed tissue. Manual DNA extraction using the PapilloCheck® DNA extraction kit was performed on the SurePath™ vials, in which the cones had been pre-fixed (group 1) as well as the PAP smears that were collected prior to the conisation (group 2). The HPV genotyping was performed using the SensoQuest Labcycler for PCR and the CheckScanner.

Conclusion: HPV 16 was found in 13 and 14 cases in group 1 and 2 respectively. The one case, where HPV 16 was found only in group 2, was a case of multiple infection with other HR-HPV types that were equally detected in both groups. Minor discrepancies between HR-HPV types were seen in 6 cases, where an extra HR-HPV type was found in 4 cases in group 1 and in 2 cases in group 2. HPV 45 was found in one case in the 1st group but missed in 2nd. HR-HPV was not detected in any group in 2 cases. The cone in one of these 2 cases was negative, and the other revealed CINII. The morphology and the immunostaining of the cones in our study were still excellent despite the pre-fixation in SurePath™ preservative. HR-HPV 16 was distributed evenly in the two groups.

Due to the good correlation between the results the SurePath™ vial seems feasible for pre-fixation of cone biopsies and the fluid seems feasible for HPV genotyping.
**ProExC™ RELIABLY DISTINGUISHES MODERATE CERVICAL DYSPLASIA**


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**Objectives:** Diagnosing cervical dysplasia by conventional hematoxylin & eosin (H&E)-based methods is limited by poor interobserver reproducibility and significant inaccuracy, especially when distinguishing cases of moderate (CIN 2) dysplasia. This is significant because current clinical management guidelines place new weight on distinguishing CIN 2 from CIN 1 and CIN 3. For example many clinicians follow CIN 2 in younger women rather than treat them with surgery. We hypothesize new molecular tools such as ProExC, or p16 plus Ki-67 immunostaining, may significantly improve our ability to reliably distinguish CIN 2.

**Methods:** 500 colposcopic biopsies were randomly selected from a series of 5000 patients with at least five years of clinical followup, including either negative serial Pap tests or surgical excision (gold standard outcome). Two pathologists independently scored each case as Negative, CIN 1, CIN 2, or CIN 3 while blinded to the original H&E-based diagnosis and five-year outcome. Diagnoses were reported based on H&E only, H&E and ProExC, H&E and p16, or H&E plus p16 and Ki67. Each staining group was scored after a sufficient washout period. Kappa statistic and test accuracy were calculated for each staining group relative to the gold standard outcome.

**Conclusions:** Sufficient tissue was available from 383/500 cases, including 45 negatives, 162 CIN 1, 100 CIN 2, and 76 CIN 3 by H&E-based diagnoses. The CIN 2 diagnosis was poorly reproducible between expert gynecologic pathologists and the original diagnosis (Table). Reproducibility improved with the aid of immunohistochemistry and test accuracy significantly improved with immunostaining. ProExC was the most reproducible method (kappa 0.57) and had the best predictive value for CIN 2.

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>CIN 2 Kappa</th>
<th>Test Accuracy (CIN2+)</th>
<th>PPV [95% CI] CIN 2 vs CIN 3</th>
<th>LR [95% CI] CIN 2 vs CIN 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E only</td>
<td>0.27</td>
<td>82%</td>
<td>62 [51-71]</td>
<td>1.9 [1-3]</td>
</tr>
<tr>
<td>p16 plus Ki-67</td>
<td>0.52</td>
<td>94%</td>
<td>79 [70-85]</td>
<td>2.5 [2-3]</td>
</tr>
<tr>
<td>ProExC</td>
<td>0.57</td>
<td>93%</td>
<td>93 [86-96]</td>
<td>10.0 [5-19]</td>
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**DIFFERENTIAL METHYLATION CONTROLS HPV16 GENE EXPRESSION DURING EPITHELIAL DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION**

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**Objectives:** The life cycle of HPV is coupled to the cellular differentiation program of the squamous epithelial host cells. Progression of acute to transforming HPV-infections is triggered by the over-expression of the E6 and E7 oncoproteins. Methylation of the viral genome may affect regulatory features that control transcription of the viral genome. Here, we analyzed the methylation pattern of HPV16 URR during squamous epithelial differentiation and neoplastic transformation and analyzed how changes in HPV URR methylation affect the viral oncogene expression.

**Methods:** 15 HPV 16 positive lesions that encompassed all stages of an HPV-infection (latent, permissive and transforming) were micro-dissected. Methylation of the HPV16 URR in cell fractions representing the basal, intermediate, and superficial cell layers, as well as from transformed p16<sup>INK4a</sup>-positive cells was analysed by bisulfite genomic sequencing. The effect of site-specific DNA methylation was analyzed using reporter gene- and gene expression assays.

**Conclusions:** In normal HPV-positive epithelial areas, all Cpg dinucleotides were consistently methylated throughout the whole thickness of the epithelium. In low grade lesions, the promoter region in the basal or intermediate cell layers contained unmethylated CpGs. In contrast, in the superficial cells most of the CpGs were methylated including the E2 and SP1 binding sites. The CpGs of the enhancer region were heavily methylated in basal cells but showed less methylation in more differentiated cells. In the 5'LCR region all CpGs were unmethylated irrespective of differentiation stage. In the majority of high grade lesions (13/15, 86,7%), consistent methylation of E2BS1 was observed. Methylation of E2BS1 leads to 4-6 fold activation of the early p97 promoter. These data support the hypothesis that differential methylation of Cpg dinucleotides in the HPV URR is linked to the squamous differentiation pattern of the epithelial host cells and thus potentially be involved in the regulation of viral gene expression and replication.
**FC 6-3**

THE EFFECT OF HPV 16/18 E6 ON TERT PROMOTER METHYLATION IN CERVICAL CANCER CELLS

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**Objective:** HPV oncoprotein E6 activates telomerase reverse transcriptase (TERT) expression and causes cellular immortalization. But it remains unclear if E6 could affect TERT transcription by DNA methylation. In this study we explored the methylation status of TERT promoter in cervical cancer cell lines and the changes of it after E6 was being silenced by RNAi.

**Methods:** Three kinds of cervical cell lines, HPV16 positive Caski and Siha, along with HPV18 positive Hela, were taken to analyze the methylation status of TERT promoters by methylation-specific polymerase chain reaction (MSP) and bisulfite sequencing (BS). Stealth RNAi was transiently transfected to these cell lines to silence the HPV16/18 E6 genes, and then the changes of mRNA level of TERT and the status of promoter methylation were examined.

**Results:** Hypomethylation status around the transcription start site (-156~+162bp) of TERT was related to its transcription. After transfection with Stealth RNAi, the levels of HPV16/18 E6 and TERT mRNA were greatly decreased. The methylated CpG around the transcription start sites in Caski and Siha cells were statistically increased (respectively P=0.016, P<0.001). But there were no significant difference in Hela cells (P=0.128).

**Conclusions:** Hypomethylated CpG in -156~+162bp around the transcription start site allow for the expression of TERT in cervical cancer cells. HPV16 E6 can promote TERT transcription through demethylating the DNA sequence around the transcription start site of TERT in cervical squamous cancer cells.

**FC 6-4**

EXPRESSION OF THE P16\(^{INK4A}\), SEVERITY OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION: ON-GOING STUDY

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**Objective:** The aim of this study is to evaluate the correlation between the expression of p16\(^{INK4a}\) (p16) in cervical intraepithelial neoplasia (CIN) and high-risk human papillomavirus (HPV) infection.

**Methods:** Until now, from September to December 2010, we analyzed 32 patients treated for CIN at Mauriziano Hospital, Department of Gynecological Oncology, University of Torino. Patients are treated by loop electrical excision procedure (LEEP) or by surgical conization. The expressions of p16 is evaluated by immunohistochemical methods. HPV genotyping is used to detect high-risk HPV.

**Results:** We found 15 patients with CIN1 and 17 patients with CIN2-3 lesion. P16 was over-expressed in 2/15 CIN1 and in 12/17 CIN2-3. Over-expression of p16 (p=0.001) was significantly associated with CIN 2-3 and with high-risk HPV infection (p=0.002).

**Conclusion:** CIN2-3 and high-risk HPV infection are significantly related to the over-expression of p16. Therefore, in the diagnosis of CIN and high-risk HPV infection, p16 can be a useful biomarker. These findings need validation increasing number of cases.
Objectives: Cervical cancer is one of the most common cancers in women worldwide, being mainly caused by infection with a high-risk group of HPVs. The Raf kinase inhibitory protein (RKIP) negatively regulates the Raf/MEK/ERK pathway by interfering with the activity of Raf-1. Down-regulation of RKIP has been associated with tumor progression and metastasis in several human neoplasms. With this work, we aimed first to assess the role of RKIP in the clinical outcome of cervical cancer patients. Secondly, we aimed to assess in vitro the biological function of RKIP downregulation in cervical cancer.

Material and methods: In the present study, 259 uterine cervix tissues, which included 45 cervicitis, 90 cervical intraepithelial lesions (SIL), 41 adenocarcinomas (AC), 70 squamous cell carcinomas (SSC) and 13 adenosquamous carcinomas (ASC), were analyzed for RKIP expression by immunohistochemistry. We found that RKIP expression significantly decreases during malignant progression, being highly expressed in non-neoplastic tissues (cervicitis: 64.5%; LSIL, 44.7%; HSIL, 53.5%) and expressed at low levels in the invasive carcinomas (SCC, 12.9%; AC, 19.5%; and ASC, 15.4%). It were not found correlations between RKIP expression and clinic-pathologic data.

Afterwards, we performed in vitro downregulation of RKIP by short-hairpin RNA in Hela cells. The cell survival, anchorage-independent growth, and wound-healing migration assays showed that RKIP abrogation was associated with increased cellular proliferation, anchorage-independent growth and migration in this cervical cancer cell line.

Conclusions: We observed for the first time in cervical cancer, that RKIP expression is lost during malignant progression of these tumours. Importantly, we demonstrated in vitro that RKIP downregulation is associated with features of tumour aggressiveness such as higher cell growth, proliferation and migration.

In conclusion, our results indicate RKIP as a marker of tumour aggressiveness and progression in cervical cancer.

Co-expression of P63, Ki-7, P16, and Human Papillomavirus (HCII) in Early Cervical Neoplasia

Objective: p63, a homologue of the tumor suppressor gene p53 is thought to be a marker of immature cell phenotype. Its role and implication in early cervical neoplastic process remains unclear. This study investigated the co-expression of this gene product with the cell proliferation marker Ki-67, the16 protein and the presence of Human Papillomavirus in early cervical neoplasia

Methods: Ninety-nine biopsies from 73 patients were chosen from a total of 1850 patients enrolled in trials examining emerging technologies for cervical cancer management. At least two independent blinded reviews were performed for each biopsy. Endocervical samples were collected and tested for HPV using the Hybrid Capture II method (Digene). Five adjacent 4μm-thick sections were respectively stained with H&E, Feulgen-Thionin, Ki67, p16 and p63. The diagnostic area delineated by our study pathologist on the H&E section was also assessed on the other four sections. We used a similar grading system to measure the degree of expression of the three markers within the different epithelial layers: negative staining, parabasal, lower third, middle third and full thickness staining.

Results: The ninety-nine lesions were classified as normal (n=21), atypia (n=19), CIN1 (n=23), CIN2 (n=18) and CIN3 (n=18). The proportion of lesions showing extended p63 expression in the intermediate and superficial layers of the epithelium increased from normal specimen to CIN 3 lesions. In 10 of the 11 CIN 1 lesions, the expression of p63 was limited to the lower third of the epithelium. Five of the 10 CIN3 lesions were negative for p63 but p16 and ki67 were expressed up to the superficial layer. There was no correlation between the degree of p63 expression and the HPV status. Overall, we observed a large range of co-expression patterns of the three markers within the different diagnostic grades.

Conclusion: Our study illustrates the complexity of the p63 involvement in the early cervical neoplasia. Never the less we believe that the analysis of the co-expression of different biomarkers in the early steps of the cervical neoplastic growth will create a more complete picture of this dynamic process.
Objective: The majority of low grade squamous intra-epithelial lesions of the cervix do not progress to high grade lesions or malignancies. The current tests commonly employed in cervical screening (traditional or thin-layer cervical smears cytology; high risk HPV panels and HPV genotyping) do not clearly distinguish which low-grade lesions (LSIL) are destined to progress to high grade lesions or malignancies. Studies in the molecular cytogenetics of cervical cancer have identified a variety of chromosomal aberrations in cervical precursor lesions, the most consistent modifications being detected in the long arm of chromosome 3, where the 3q26 region harbors the human telomerase gene involved in telomeres maintenance (1). Consistent data demonstrated that gene amplification in the 3q26 region are temporally associated with the integration of HPV DNA into the host genome, which constitutes a crucial step in the over production of E6 and E7 oncoproteins that play an important role in the development of cervical cancer (2). Finally, recent studies demonstrated a correlation between the gain in 3q26 copy number and the severity/stage of cervical disease and examined the potential utility of 3q26 gain as a predictor marker of progression of low grade squamous intraepithelial lesions of the cervix (3).

Methods: Slides coated with cervical cells are hybridized with a probe for the chromosome 3q26 region and analyzed using Ikoniscope® Digital Microscopy System (Ikonisys). The entire sample is scanned and the total number of nuclei is ordered based on the number of 3q26 signals. Samples are considered positive when at least 2 nuclei with at least 5 copies of 3q26 are observed.

Results: Our experience in FISH utilization will be presented and a report case will be described in details: a marked progression in both severity and extent of a lesion from atypical squamous cells to CIN 2 - CIN 3 over the span of one year confirmed the FISH potential in predicting progression. The initial gain of 5 copies of 3q26 in only 3 nuclei (0.5%) in this patient’s first cervical smear, in presence of a negative colposcopy, was a clear indication of the significance and sensitivity of this degree of gain, even in a small number of cells at a low level of disease. The subsequent gain of 3q26 one year later in the very large number of 264 nuclei (50%) reflected both the severity and extent of disease progression as confirmed, in this case, by the positive colposcopy.

VIRAL LOAD, PHYSICAL STATUS, AND E6/7 mRNA EXPRESSION OF HUMAN PAPILLOMAVIRUS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Objectives: The aim of the present study was to determine human papillomavirus (HPV) load and physical status, especially HPV-16, in different types of head and neck squamous cell carcinomas (HNSCCs) using freshly frozen HNSCC samples.

Methods: A total of 184 specimens were obtained from 184 patients with HNSCC. The prevalence of HPV DNA was also determined in tonsils resected from 47 control patients (age, ≥18 years) who underwent tonsillectomy for chronic tonsillitis. After DNA extraction, PCR was performed, followed by direct sequencing to identify the HPV type in each specimen. The viral load and the physical status of HPV-16 were then determined by real-time PCR of E6 and E2 genes. After RNA extraction, expression of E6/7 mRNA was also analyzed by reverse-transcription PCR.

Results: HPV was detected in 54 HNSCC samples (29.3%), and the HPV genome was observed in 50.0% of cancers of the oropharynx, 32.3% of the oral cavity, 30.8% of the nasopharynx, 16.4% of the hypopharynx, and 15.4% of the larynx. In the oropharynx, 69.6% of tonsillar carcinomas were HPV positive. The viral copy numbers in tonsillar carcinoma specimens were significantly higher than in non-tonsillar HNSCCs. Analysis of viral physical status in HPV-16-positive samples demonstrated the presence of either the integrated or mixed forms in 75.6%. Reverse-transcription PCR detected E6/7 mRNA transcription in 13 of 51 (25.5%) HPV DNA-positive cases. High HPV-16 DNA load was significantly correlated with expression of E6/7 mRNA of HPV-16.

Conclusions: The results of the present study demonstrate a significant relationship between HPV and HNSCC, especially for cancers of the palatine tonsil. According to previous studies from mainland Japan and Korea, our HPV-16 copy number of tongue and pharynx carcinomas in Okinawa, a southern island of Japan, was relatively higher than those reports. Our results might reflect the fact that the HPV viral loads varied within a wide range among geographically adjacent areas, such as mainland Japan and Korea.

GENETIC VARIANTS OF PRE-MIRNA GENES AS A PREDICTOR OF HPV16 STATUS AND SURVIVAL OF OROPHARYNGEAL CANCER PATIENTS

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Objective: To identify non-tumor biomarkers for prediction of tumor HPV status and prognosis of patients with squamous cell carcinoma of the oropharynx (SCCOP), we evaluated the association of single nucleotide polymorphisms (SNPs) in pre-miRNAs with HPV16 status and survival for SCCOP patients.

Methods: We analyzed HPV16 status in tumor specimens and genotyped four SNPs in pre-miRNAs (hsa-mir-146a rs2910164 G>C, hsa-mir-149 rs2292832 G>T, hsa-mir-196a2 rs11614913 C>T, and hsa-mir-499 rs3746444 A>G) in 209 SCCOP patients. Unconditional logistic regression models were used for calculation of odds ratio (OR) and 95% confidence intervals (CIs), and Kaplan-Meier analysis and Cox proportional hazards regression were used to evaluate associations with risk of death or recurrence.

Conclusion: Statistically significant associations with HPV16-positive SCCOP were found for hsa-mir-149 rs2292832 and hsa-mir-199 rs3746444 and with survival for hsa-mir-146a rs2910164 and hsa-mir-196a2 rs11614913. Compared with those with corresponding variant genotypes, the hsa-mir-149 CC and hsa-mir-199 TT wild-type genotypes were significantly associated with HPV16-positive tumor status (adjusted OR, 2.4; 95% CI, 1.2-4.6 and adjusted OR, 2.0, 95% CI, 1.0-3.9), respectively. Patients having hsa-mir-146a rs2910164 GG and hsa-mir196a2 rs11614913 CT/TT genotypes had significantly better overall, disease-specific, and disease-free survival compared with those having the corresponding CG/CC and CC genotypes, respectively. Furthermore, these genotypes were significantly associated with approximately 70% reduced or increased risk of death or recurrence after adjustment for important prognostic confounders including HPV status, smoking, and stage. These findings suggest that pre-miRNA polymorphisms may predict HPV16 tumor status and may be prognostic biomarkers for SCCOP.
**HUMAN NAPILLOMAVIRUS INFECTION AND P16\(^{INK4a}/KI-67\) CO-EXPRESSION IN THE HEAD AND NECK SQUAMOUS EPITHELIUM**


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**Objectives:** Head and neck squamous cell carcinoma (HNSCC) caused by high-risk human papillomavirus (HR-HPV) infection has been acknowledged as an individual tumor entity. In transformed and thus proliferating cells of the uterine cervix the tumor suppressor p16\(^{INK4a}\) is strongly expressed by a HR-HPV induced mechanism, while strong p16\(^{INK4a}\) expression in normal epithelium is restricted to cell cycle arrested, senescent cells. Accordingly, cervical HR-HPV transformed cells can be identified by simultaneous detection of p16\(^{INK4a}\) and the proliferation marker Ki-67 in the same cell. In the present study we determined the prevalence of HR-HPV infection in head and neck samples of varying differentiation and correlated it to the p16\(^{INK4a}/Ki-67\) co-expression profile to verify whether HR-HPV transformed cells in the head and neck feature similarities to those in the anogenital tract.

**Methods:** The co-expression profile of p16\(^{INK4a}/Ki-67\) in normal (n=50), premalignant (n=36) and malignant (HNSCC; n=98) head and neck tissue was evaluated using p16\(^{INK4a}/Ki-67\) immunohistochemistry. 14 HR-HPV genotypes were detected applying Luminex technology.

**Conclusions:** 8% (4/50) of normal, 38.9% (14/36) of premalignant head and neck samples and 8.2% (8/98) of HNSCC were HPV positive. In normal tissue no p16\(^{INK4a}/Ki-67\) co-expressing (p16\(^{INK4a}/Ki-67\)-positive) cells were detected. p16\(^{INK4a}/Ki-67\)-positive cells were identified in 21.4% of HPV-positive and 18.2% of HPV-negative premalignant samples and in 62.5% of HPV-positive and 3.3% of HPV-negative HNSCC.

Co-expression of p16\(^{INK4a}/Ki-67\) in the very same cell therefore is a hallmark for neoplastic conversion as it is found in premalignant and malignant samples and in HNSCC but not in normal tissue. Our findings indicate the dependency of p16\(^{INK4a}/Ki-67\) co-expression on infection with HR-HPV in HNSCC. A small percentage of samples however show p16\(^{INK4a}/Ki-67\) co-expression while being HPV-negative. Interestingly, these samples were more frequently found in premalignant tissue necessitating further analysis on the relevance of this finding with respect to biological and clinical behaviour of these lesions.

**PRIMARY SQUAMOUS CELL CARCINOMA OF THE TONSILS, HUMAN NAPILLOMAVIRUS DETECTION, TYPING AND RELATION TO P16\(^{INK4A}\) CORRELATED TO OVERALL SURVIVAL.**

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**Introduction:** Tonsillar squamous cell carcinoma (SCC) is the most common oropharyngeal cancer in Denmark, and recent published data show that the incidence has increased three-fold over the last 30 years. It is well-known that use of tobacco and increased alcohol consumption are risk factors for developing tonsillar cancer, but during the last decades studies identifying HPV infection as a risk factor have emerged. Data on the subject are still sparse and many studies have used p16 as a surrogate marker for HPV infection. In the present study we try to elucidate the prevalence of HPV using a genotyping assay and investigate the correlation with p16 expression and the relation to overall survival.

**Material:** All patients diagnosed with SCC of the tonsils during 2000-2009 at Department of Clinical Pathology, Sygehus Lillebælt were retrieved from the archives. Sixty-four patients were found, and 58 had formalin fixed paraffin embedded tissue available for further analysis. Overall survival was recorded from the clinical records.

**Method:** HPV analysis with genotyping.

Briefly, DNA was extracted from paraffin embedded tissue, and HPV was detected using the Linear Array genotyping assay, which is designed to detect 37 HPV types. p16\(^{INK4A}\) Immunostaining was performed using the automated immunohistochemical system Autostainer Link 48 (AS480, DAKO, Glostrup, Denmark). p16\(^{INK4A}\) antibody was obtained from Santa Cruz (JC8).

**Results:** Thirty-seven (64%) patients were positive for HPV and 21 (36%) patients were negative by Linear Array, 35 had HPV 16, 1 had HPV 35 and 1 had HPV 59. 35 patients were HPV positive and p16 positive, 19 patients were HPV negative and p16 negative, 2 patients were HPV negative and p16 positive and 2 patients were HPV positive and p16 negative. The concordance between HPV and p16 was 93%.

HPV positive patients had a significant better overall survival than HPV negative patients (p<0.004). There was no significant age or gender difference between the two groups.

**Discussion and conclusion:** This study indicates that Linear array are a feasible method for HPV detection and genotyping in patients with tonsillar squamous cell carcinomas. Although the study only includes 58 patients it offers important data on how often HPV may be involved in tonsillar squamous cancer, as 64% patients had either HPV 16, 35 or 59. Sixty percent had tonsillar squamous cell carcinoma and HPV 16, and only a prospective and larger study will be able to evaluate, if these cases can be rescued, if the population is vaccinated against HPV.
**ORAL HPV PREVALENCE AMONG HIV-1 INFECTED MALES AND FEMALES IN ITALY**

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**Objectives:** To evaluate type-specific prevalence of oral HPV infection in a group of about 100 Human Immunodeficiency Virus (HIV) infected subjects; correlation with anatomo-clinical and immuno-virological parameters will be also analyzed.

When available, samples collected in the subsequent 12-24 months will be analyzed.

**Methods:** DNA has been obtained from saliva samples from HIV-positive male and female patients included in a study on Human Herpesvirus 8 (HHV8) and Kaposi’s sarcoma. A significant proportion of males are men who have sex with men (MSM), and the vast majority are of Caucasian origin. HPV sequences are searched by PCR with MY09/MY11 primers, or by nested PCR with GP5+/GP6+ primers as second step. Typing is accomplished by restriction fragment length polymorphism analysis or by direct sequencing. HHV8 sequences are detected and quantified by nested PCR and real-time PCR, respectively.

**Conclusions:** Preliminary data obtained in a small group of samples indicate an oral HPV prevalence of about 30% (8/30). The majority of HPV-positive patients have infections with high-risk types. HPV prevalence is higher (6/13, 46%) among MSM, and infrequent among women. No correlation appears to exist between HPV and HHV8 infections.

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**HIGH-RISK HPV DNA IN LYMPH NODES IN CERVICAL CANCERS PATIENTS**

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**Objectives:** Metastatic involvement of lymph nodes is the most important prognostic parameter in cervical cancer. Still, approximately 15% of patients with negative nodes experience recurrence. The presence of HPV DNA in histology-negative pelvic nodes is evaluated as a marker of subclinical metastatic spread.

**Methods:** Patients with early-stage cervical cancer referred for radical surgical treatment and patients with locally advanced cervical cancer referred for staging paraaortic lymphadenectomy were enrolled in the study. Control group was consisted of ovarian cancer and endometrial cancer patients, who underwent radical surgery with systematic lymphadenectomy. Cytobrush technique was used for sample collection from the fresh tissue of cervix and pelvic or paraaortic lymph nodes to avoid any loss of material for histology.

**Conclusions:** Altogether, 49 early-stage, 23 locally advanced cervical cancer patients and 10 non-cervical cancer patients in control group were enrolled in the study. High-risk (HR) HPV was identified in the tumor in 91.8% early stage and 100% advanced cancers, in the sentinel node or other pelvic nodes in 49.9% patients and in paraaortic nodes in 88.2%. Among the 10 HR HPV genotypes detected, HPV 16 was the most frequently represented in both the tumor and the lymph nodes (66.7% and 71.4%, respectively). All metastatic lymph nodes were HPV positive with strict consistency between genotypes in the tumor and positive nodes. There were no HPV positive cervical smear or lymph node in the control group. The presence of HR HPV DNA in a sentinel node had a positive predictive value for metastatic involvement of lymph nodes in our study. This could be considered as a sign of an early subclinical metastatic spread and thought as a prognostic factor; however, the clinical value has to be evaluated through a longer follow-up.
Cervical cancer has been the cause of death for women all over the world. Extensive research is ongoing with the aim of reducing the incidence of cervical cancer and for designing therapeutic strategies for its prevention. Persistent HPV infections, mostly by sexual transmission, are recognized as the cause of essentially all cervical cancers. Hence, immunization with the HPV vaccine has been proposed to be a major means of cervical cancer prevention and is presently under trial. The HPV vaccine is most effective before a person is infected with an HPV, which is why the vaccine has been recommended for girls as young as nine, up to the age of 26, and tests are also under way to see if it is effective for women over that age. The vaccine protects women against four HPV types that cause 70% of all cervical cancers. Studies are also undertaken for scientific evidence on the comparative efficacy to induce and sustain immunogenicity, immune memory, and prevent infection by HPV types included in the vaccine. However, 30% of cervical cancers remain impervious to the beneficial effects of HPV vaccination and thus, alternate strategies, especially from natural sources, are also under investigation for the treatment of affected individuals.

Artemisinin (ART), a naturally occurring sesquiterpene lactone from Artemisia annua, has recently been suggested to have anti-cancer effects in vitro and in vivo, although the mechanisms for its anti-tumor activities have not been fully elucidated. Thus, the major objective of this work was to analyze the effects of ART on cervical disorders caused by the human papillomavirus (HPV) in vitro, using a cervical cancer cell line, ME-180. ME-180 cells were treated with various doses of artemisinin at different time intervals. Cell proliferation and toxicity were determined by MTT viability assay. Apoptosis and cell cycle progression were evaluated using flow cytometry assay. Changes in expression of telomerase components, cell cycle regulatory genes, ER and VEGF were analyzed at both mRNA and protein levels by RT-PCR, western blot analysis and immunocytochemistry. The results indicated that ART exhibits cytotoxicity in cervical cancer cell lines and triggered G1/S cell cycle arrest in a dose and time-dependent manner. ART treatment induced early apoptotic cell death in ME-180 cells, along with significant downregulation of cyclin D1, CDK4, ER and VEGF at the mRNA transcript and protein levels, and concomitant upregulation the cell cycle inhibitor p21Waf1/Cip1 at the mRNA transcription level. Inhibition of cell proliferation was further supported by significant downregulation of telomerase components, viz., the hTR and hTERT transcripts. The results suggest that artemisinin leads to cell cycle arrest, induces apoptosis and significantly reduces the expression of genes related to cellular proliferation and angiogenesis in cervical cancer cells. Since ART does not exhibit any toxic effect on normal cells, it might be developed as a potential chemotherapeutic agent for the treatment of human cervical cancer.

**FINNISH MEDICAL BIRTH REGISTRY DATA DURING 1998-2009: LEEP CONISATION AND THE RISK FOR PRETERM BIRTH**

**Objectives:** To study whether the increasing severity of cervical intraepithelial neoplasia (CIN) of LEEP cone (loop electrosurgical excision procedure) correlates with the risk for preterm birth. We also wanted to study if time period since LEEP or repeat cone has impact on preterm birth rate.

**Methods:** Retrospective register-based study from Finland during 1997-2009. We gathered information from the Hospital Discharge Register (HDR) and we linked the information with data from the Finnish Medical Birth Register (MBR) using personal identification numbers. The study population consisted of 20,011 women who had LEEP conisation during 1998-2009 and a subsequent delivery during 1997-2009. Our control population consisted of women in MBR with no previous LEEP treatment (n=430,975). Main outcome measure was preterm birth (< 37 gestational weeks) rate.

**Results:** The risk for preterm birth was increased after LEEP conisation (OR 1.82, CI 1.62-2.03). In primiparous women this risk was slightly lower OR 1.61 (CI1.42-1.83). Repeat LEEP was associated with almost three-fold risk for preterm birth with OR 2.71 (CI 1.98-3.69). Increasing severity of CIN did not correlate with preterm birth rate. LEEP treatment for carcinoma in situ or microinvasive cancer, however, increased the risk for preterm birth three-fold (OR 3.25, CI1.92-5.50). The increased risk was observed also for other HPV-related non-CIN-lesions (OR 2.55, CI 1.72-3.78). Time period since LEEP was not associated with risk for preterm birth. Adjusting for maternal age, parity, socio-economic status or marital status, urbanism and previous preterm birth did not change the results.

**Conclusion:** The risk for preterm birth was increased after LEEP procedure, but not by severity of CIN. Repeat LEEP was most risky and should be avoided especially among fertile-aged women.
PERFORMANCE OF HIGH-RISK HPV TEST IN THE SURVEILLANCE OF PATIENTS TREATED FOR HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA.

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Objectives: the aim of our study is to evaluate the accuracy of high-risk human papilloma virus (HR HPV) DNA detection as predictor of residual or recurrent cervical intraepithelial neoplasia (CIN) after treatment for high grade CIN.

Methods: we carried out a retrospective study of 172 patients treated by loop electrosurgery excision procedure (LEEP) and conization for High Grade Cervical Intraepithelial Neoplasia (CIN2+). After treatment the patients were followed up (mean and median 12 months, range 3-29) with cervical cytology (ThinPrep™ traditional lecture, and Surepath™ FocalPoint LGS™ lecture) combined with HR-HPV DNA test (hc2 Qiagen™). Three parameters were taken into account. The status of the resection margins (positive-in sano) by histology at treatment time, cytological diagnosis (NILM –ASC+, TBS 2001), and the presence of HR-HPV (hc2 positive RLU ≥ 1.00 – negative RLU <1.00). The reference standard for residual/recurrent cervical neoplasia was CIN1+ histological diagnosis. Sensitivity, Specificity, Positive and Negative Predictive Values, Diagnostic Odds Ratio (DOR calculated as LR+/LR-), probability p were evaluated for each individual variable.

Results: After treatment the Patients were followed with colposcopy, cytology and HR-HPV test. During the follow up period 62 patients underwent histological assesment. 53 (30.8%of the total population) of these had residual/recurrent CIN1+ disease (CIN1 n.29, CIN2 n.12, CIN3 n.12). DOR and p summarize the performance of each variable. Resection margins showed DOR 3.92, p 0.061; cytology showed DOR 2.40, p 0.220; HR-HPV showed DOR 21 p 0.002. Moreover, the relative sensitivity was: HPV/margins 1.18, HPV/cytology 1.25, Cytology/margins 0.94.

Conclusions: The HR-HPV test was showed to be a better predictor of residual/recurrent CIN1+ disease than cytological diagnosis and resection margins in the follow up of Patients treated for High Grade CIN.

IMPROVED DETECTION OF HIGH GRADE CIN IN WOMEN UNDERGOING COLPOSCOPY USING ELECTRICAL IMPEDANCE SPECTROSCOPY

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Objectives: To determine if use of an Electrical Impedance Spectroscopy (EIS) device improves detection of high grade CIN. We report the results of our study, establishing a baseline performance for colposcopy and performance characteristics of the EIS device.

Methods: 191 women with abnormal cervical cytology were recruited at two colposcopy clinics. Referral cytology was severe (64), moderate (30) and mild dyskaryosis (41), borderline (45), ASC-H (7), ?invasive (1), glandular neoplasia (2) and AGC-US (1). 72% of borderline and mild dyskaryosis were positive for high-risk HPV types. Mean age was 34 years (23-60 years), 7 patients were post-menopausal (3.6%). The study population was Caucasian (92.1%) African/Black (4.7%), Indian/Asian (2.1%) and Oriental (1%).

The EIS device – APX100 – was used to take 12 readings from the cervix, including the TZ, before and after the application of 5% acetic acid. Acetic acid was re-applied before completing the colposcopic examination and biopsies taken per local protocols. Colposcopic examinations, including APX 100 positioning, were video recorded to produce a colposcopic diagnosis for each APX100 reading site and a measure of the accuracy of colposcopy directed biopsies. EIS spectra are compared with reference modelled spectra to derive a measure of probability that high grade CIN is present at a site. Probability values are compared with the colposcopic diagnosis to determine the agreement between the two methods. Receiver operating characteristic (ROC) curves were derived to discriminate high-grade CIN (>CIN1) from all other cervical tissue types both pre- and post-acetic acid (AA) at each reading site. Areas under the curve (AUC) were 0.77 (pre AA) and 0.79 (post AA). Comparison of these curves showed no significant difference, indicating that application of AA does not produce a large change in spectra. PPV for colposcopic impression was 69% using a cut-off of >CIN1 as high-grade CIN

Conclusions: This study has confirmed and extended our previous work demonstrating EIS can be used to discriminate between high-grade CIN and other tissues in the cervix. The next stage is to use the APX100 device with colposcopy to improve the selection of biopsy sites and improve the PPV of the procedure.
PENISCOPIC EVALUATION OF THE MALE PARTNERS OF WOMEN TREATED FOR HIGH-GRADE CERVICAL LESIONS

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Objectives: To evaluate the role of peniscopic examination in detecting HPV lesions from male sexual partners of women treated with cervical excision for a histologically confirmed high grade cervical lesion.

Methods: Male partners of 84 women treated for a high-grade cervical lesion were investigated by clinical evaluation, peniscopy before and after application of acetic acid (5%), and biopsy of acetowhite lesions.

Conclusions: 63 patients had a negative peniscopy. In 6 patients condylomata acuminata of the penis, pubis and/or the perineal area were detected and treated with CO2 laser vaporization. A suspicious white epithelium of the glans was biopsied in 15 cases: in 12 patients histology revealed a penile intraepithelial neoplasia grade 1 (PIN 1), while in 3 patients with an irregular punctuation pattern the biopsy revealed a penile intraepithelial neoplasia grade 3 (PIN 3). PIN 1 was not treated, while PIN 3 was treated with laser (one of these patients had a relapse and was treated again). In 21 patients pearly penile papules – a harmless anatomical condition – were described.

Peniscopy is a valuable tool in identifying “high-risk males” who may harbour HPV lesions, but its role in routine clinical practice is doubtful, since the value of treating male partners of HPV infected women is unclear.


INTRODUCING A NEW DIAGNOSTIC MODALITY INTO THE STANDARD OF CARE

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Objectives: To determine the projected outcomes of introducing a new diagnostic modality for cervical disease detection. The results of a seven-center pivotal study with two year follow up was used to determine the impact that multimodal hyperspectroscopy (MHS) could have if implemented as a test to triage women at risk for cervical dysplasia.

Methods: In the seven-center pivotal study, 1,607 women at risk for cervical neoplasia were tested using MHS (LightTouch, Guided Therapeutics, Inc. Norcross, GA), including 126 with HSIL Paps, 71 with ASC-H Paps, 737 with LSIL Paps, 523 with ASC-US/AGC-US Paps, one with no Pap results and 149 with normal cytology but were at risk for other reasons including positive HPV results and previous dysplasia. Of these 1,607 women, 804 had follow-up results of up to two years.

Conclusions: Of the 1,607 women tested, 160 were excluded prospectively from analysis because they were either training subjects, had uncertain histopathology and/or device/user errors. For the 1,447 women with evaluable data, sensitivity of MHS was 91.0%, specificity was 38.8%, negative predictive value was 93.9% and positive predictive value was 25.8%. Based on the 804 women with follow up, the current standard of care delayed diagnosis of CIN2+ in 46 of 134 women, a false negative rate of 34.3% (sensitivity = 65.7%) which is roughly equivalent to the false negative rate reported in the ASC-US LSIL Triage Study (ALTS). MHS correctly identified 121 of these 134 cases (sensitivity = 90.3%) at the time of the initial visit (p < 0.0001). In addition, of the 279 women found to have a normal cervix after follow up, MHS diagnosed 112 (40.1%) as free of CIN2+ disease, while of the 329 women found to have CIN1 after follow up, MHS diagnosed 118 (35.9%) as free of CIN2+ disease. Extrapolation of these results to the US population at large would result in earlier identification of CIN2+ in over 200,000 women and a reduction of biopsies on normal or low grade dysplastic tissue in nearly 1.2 million women annually. MHS is a cost effective, non-invasive test that provides an objective result immediately after a one- minute scan of the cervix is completed. Implementation of MHS could potentially result in the earlier identification of hundreds of thousands of women in the United States and Europe with CIN2+. In addition, MHS could potentially reduce unnecessary post screening procedures, including biopsies, for over one million women annually.
HIGH RISK HPV TYPES IN WOMEN WITH ABNORMAL CERVICAL CYTOLOGY IN A SOUTH AFRICAN POPULATION WITH A HIGH PREVALENCE OF HIV

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Objectives: The prevalence of HPV infection and cervical cancer in Sub-Saharan Africa has increased dramatically over the past decade due to the high burden of HIV infection in this region. Newly proposed South African screening guidelines recommend the option of primary screening with HPV genotyping and then more intense follow-up of those women infected with the top cancer causing HPV types. The distribution of high risk (hr) HPV in women with abnormal cytology in our study was compared to that found in regions with a lower HIV prevalence here in South Africa and the developed world.

Methods: Population based cervical cancer screening was performed on 1089 patients in primary health care clinics in the Pretoria region of South Africa. Conventional pap smears and HPV DNA genotyping was performed on cervical swab and tampon collected specimens using the Linear Array HPV Genotyping test (Roche Molecular Systems®). The HPV prevalence and genotype distribution in the different cervical lesions were compared in the 197 women who had abnormal pap smears.

Conclusions: Cervical hrHPV was detected in 80% (12/15) of ASCUS lesions, 93.8% (30/32) of LSIL, 98.0% (144/147) HSIL lesions and 100% (3/3) cervical carcinomas. In the subset of women with HSIL and cervical cancer the most prevalent HPV types were 16 (32.7%), 51 (26.7%), 58 (25.3%), 35 (24.0%), 33 (20.7%), 66 (18.0%) and 18 (17.3%). The increased prevalence and dominance of HPV types in addition to those traditionally described (i.e. 16, 18 and 35) may have implications for screening follow-up and vaccination strategies in regions with a high HIV prevalence.

RESULTS OF 1206 HIV–INFECTED PATIENTS SCREENED FOR ANAL LESIONS (CONDYLOMAS, DYSPLASIA AND CANCER).

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Objectives: Systematic anal screening is recommended in France in HIV-infected patients due to the increase in anal cancer. Results of anal screening implementation in real life are unknown. We describe the results of screening of this population in a French University Hospital.

Methods and results: During 6 years (2003 to 2009), anal lesion screening was systematically proposed to HIV-infected outpatients. Systematic perianal and endoanal examination with an anoscopy was performed, such as biopsy to confirm the diagnosis and identify dysplasia. HIV-risk factors, sexual behaviors, previous history of genital condyloma and/or sexually transmitted diseases, CD4 cell count, CD4 nadir, and HIV-RNA were collected. Among the 1206 screened patients (701 homosexual and 247 heterosexual men, 258 women), 307 (25%) had anal condylomas (34% among homosexuals and 14% for the others) ; 122 (10%) had low grade associated dysplasia (AIN1) and 86 (7%) high grade (AIN2-3). Seven anal cancer were diagnosed (6 epidermoids and 1 lymphoma). No clinical and biological studied factors were associated with anal risk cancer or high grade dysplasia. In multivariate analysis, receptive anal intercourse (OR=3.03 [2.06-4.47]), CD4 cell count below 200/mm3 (OR=2.54 [1.71-3.78]), white individuals (/ black) (1.88 [1.17-3.01]), history of HPV-related lesion (1.84 [1.35-2.51]), and age below 45 years (OR=1.56 [1.16-2.11]) were independently associated with anal lesions (condylomas and dysplasia).

Conclusions: Anal screening with standard anoscopy in a large HIV-infected patients of a French University Hospital revealed a high rate of high grade dysplasia and anal cancer (6/1000). Anal HPV lesion associated factors belonged to sexual behaviors and patients’ history and characteristics. Patients with these characteristics should be screened for anal cancer.
PILOT STUDY OF HPV PREVALENCE AND CERVICAL EPITHELIAL NEOPLASIA AMONG HIV-INFECTED WOMEN IN YUNNAN PROVINCE, CHINA

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Objectives: The burden of HPV and cervical neoplasia among HIV-infected (HIV+) women in China has not been reported. We conducted a cross sectional study among HIV+ women in Yunnan Province in southwest China, an area with high HIV prevalence and limited access to healthcare.

Methods: HIV+ women underwent screening with cervical cytology and HPV detection by Hybrid Capture-2 assay, followed by diagnostic colposcopy. Women with cytological or colposcopic abnormalities underwent cervical biopsy. We investigated HIV-HPV co-infection, CIN prevalence, and associations with HIV-related clinical and laboratory parameters using logistic regression analysis.

Conclusions: The mean age of the 95 HIV+ participants was 33.5 years, median CD4+ cell count was 487/µL (interquartile range: 321-617), two-thirds (58%) were currently on antiretroviral therapy (ART), 20/95 (21%) had abnormal (ASC-US+) cytology, and 7/80 (8.8%) had colposcopic-histopathologically proven CIN2+ lesions. Over half (41/95: 54%) were co-infected with carcinogenic HPV. HPV co-infection was higher in women with abnormal (ASC-US+) cytology than women with normal cytology (crude OR: 8.0, 95%CI:2.4,26.5), and higher in women with colposcopic-histopathologically proven CIN2+ lesions than women with ≤CIN1 (crude OR: 8.1, 95%CI:0.9,71.0). Women with CD4+ cell count <350/µL had higher CIN2+ prevalence than women with CD4+≥350/µL, after adjusting for current ART status (adjusted OR: 6.5, 95%CI:1.1,37.6). Overall, HIV+ women in Yunnan, China have a high prevalence of carcinogenic HPV and precancerous lesions relative to the general Chinese population. With improving ART access, HIV+ women are expected to live longer and may be at continued higher risk for invasive cervical cancer in absence of effective early detection and treatment services.

RELATION BETWEEN ANAL AND CERVICAL HPV SCREENING AND CYTOLOGY: STUDY IN HIVneg WOMEN

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Objectives: Anal and cervical cancer resemble each other concerning site of origin, oncogenesis and histopathology. We tested the feasibility of cervical and anal HPV screening, the HPV type-specific prevalence and cytology.

Methods: HIVneg women (N=149) were consecutively sampled, using the Cervex-Brush®. All cervical slides and 49 HPVpos anal slides were pre-screened by BD FocalPointTM system and manually screened afterwards. The presence of 18 different HPV geno-types was determined with quantitative RT-PCR’s.

Conclusions: Human DNA was found in 53% of anal samples and in all cervical samples. Forty seven per cent of the anal samples showed HPV DNA vs. 24% of human-DNAneg anal samples. The corresponding cervical samples were HPV positive in 86.5 and in 88.2% respectively. Top anal types were 16, 51 and 39; top cervical types HPV 16, 51, 56 and 39. Of the 54 HPV DNApos anal samples, 57% were mono infected, 33% had 2 HPV types, and 9% had ≥ 3 infections. Two of the 7 patients with an HPVpos anal smear and an HPVneg cervical smear showed anal low grade dysplastic cells ("ASC-US"-like) and cervical ASC-US respectively. Of the remaining 42 slides, 1 patient had anal "ASC-US"-like cells, 4 patients had cervical H-SIL and 2 patients had cervical ASC-H. L-SIL was found in 3 anal samples and 14 cervical samples. Corresponding HPV types belonged to the carcinogenic HPV types.

Our patients often had multifocal HPV infections, but cytological findings were rare. It is likely that HPV vaccination will be effective in the prevention of anal cancer. Therefore anal screening is important in high-risk groups.

**Objective:** Human papillomavirus (HPV) type distribution differs between population groups, countries and regions. Viral types that are associated with pre-neoplastic lesions may also be different between HIV positive and immunocompetent women.

**Methods:** We report both the incidental (low risk) and carcinogenic (high risk) HPV types found with the DNA analysis in a cohort of 60 consecutive women presenting for treatment of cervical pre-neoplasia, to a tertiary hospital in an urban setting with a high prevalence of HIV infection. All test results were available for 60 women of which 41 women tested HIV positive and 19 HIV negative. HIV negative women had an average of two HPV types (zero to six), while HIV positive women had four types (one to ten). HPV 16 and/or 18 was present in 51% of HIV positive and 37% of HIV negative women. Three women tested negative for high risk HPV types. Immunocompetent women had on average one high risk HPV type (zero to three), while immunodeficient women had two high-risk HPV types (zero to six). Viral type, histology results, CD4 counts and treatment details will be discussed.

**Conclusion:** HIV infection contributes a large burden of cervical disease in South Africa. We confirm previously reported high numbers of both high and low risk HPV types in women infected with HIV. Without viral type cross-protection, current HPV vaccines would be extrapolated to prevent almost 47% of high-grade lesions in our population and suggest that these vaccines may even be more effective in women infected with HIV.

**CERVICAL AND ANAL PAP SMEAR SCREENING AMONG DRUG USERS LIVING WITH HIV IN MIAMI, FLORIDA.**

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**Objective:** Human papillomavirus (HPV) is associated with cervical and anal cancer. Human immunodeficiency virus (HIV) infection and cocaine use are associated with increased risk for HPV infection and associated diseases, but little is known about cervical and anal dysplasia among HIV-infected drug users. The objective of our study was to assess the rate of abnormal cervical and anal Pap smears among drug users living with HIV in Miami, Florida.

**Methods & Results:** Project HOPE (Hospital is an Opportunity for Prevention and Engagement) is a two-site study conducted in Miami and Atlanta to evaluate the efficacy of a brief prevention intervention for HIV-positive crack cocaine users recruited from 2 inner-city hospitals during their inpatient stays. At the Miami site, cervical Pap smears from women (n=29) and anal Pap smears from both women and men (n=46) were collected. The study population was 95% Black, the mean age was 47, 63% were female (29/46), 35% heterosexual males (16/46) and 2% men who have sex with men (1/46). Approximately half of the participants did not complete high school, 50% were on antiretroviral therapy and 50% had been diagnosed with HIV for more than 14 years. The median CD4 cell count was 198. Overall 69.5% (32/46) of anal Pap smears were abnormal. Among women, abnormal anal Pap smears (20/29) were almost as common as abnormal cervical Pap smears (23/29). Half of the women with abnormal cervical Pap smears also had abnormal anal Pap smears. CD4 cell count and being on antiretroviral therapy were not significant predictors of an abnormal Pap smear.

**Conclusions:** These preliminary data suggest that abnormal cervical and anal Pap smears are common in drug users living with HIV in Miami and highlight the need for further studies on prevention and screening of cervical and anal dysplasia and cancer in this population.
HPV TESTING IN PRIMARY SCREENING:
SHOULD ORGANIZED PROGRAMS BE REQUESTED BEFORE IMPLEMENTATION
A. Anttila

Outline

- In order to control for potential adverse effects and optimise the impact, well-organised evidence-based screening for cervical cancer should be provided (EC, 2003). This includes reaching an informed acceptance and high coverage of the service within the target population, and systematic quality assurance activities at appropriate levels of the programme.

- Within non-population-based service the coverage of the activity is often limited to the well-to-do population (a meaningful proportion of population not covered by the service), and a systematic external quality assurance may be missing. The activity may be running also in such target ages and with such screening intervals which would be optimal to gain a good control over the balances of potential adverse effects and benefits.

- When considering introduction of HPV screening it is important to pay attention to the programme aspects, such as: how to invite women in order to maintain a high coverage, how to build up registration and systematic quality assurance, and how to control for the increased burden of diagnostics services and pre-cancer treatments. Behavioral aspects among women, e.g. whether they adhere to a longer screening interval, need also consideration.

- An optimal strategy in settings without an organised screening programme for cervical cancer in connection with introducing primary HPV testing would be to introduce the screening protocol in an evidence-based manner within an organised programme setting.

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HPV TESTING IN PRIMARY SCREENING:
SHOULD ORGANIZED PROGRAMS BE REQUESTED BEFORE IMPLEMENTATION
“NO”

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Objective: To review the reported compliance with interval guidelines of screening systems in the United States in the fee for service sector and the HMO sector.

Methods: Comparison of published rates of compliance with HPV test interval recommendations with our experience in the Kaiser Permanente Northern California system.

Conclusions: There have been multiple reports from Dr. Mona Saraiya and her colleagues at the US Centers for Disease Control, documenting that for those physicians who include HPV testing in their screening practices, annual screening is by far the most common policy. We have recently examined our screening intervals pre and post implementation of Pap plus HPV DNA cotesting. The median rescreening interval after a second consecutive negative Pap (thereby excluding followup of recent abnormal results) was 16 months. The median rescreening interval following a negative cotest occurring between the years of 2003-2005 (followup through 2008) was 36 months. We conclude from this demonstration that provision of screening by government administered Public Health programs are not required for interval compliance, but readjustment of economic incentives to emphasize outcomes rather than payment for specific activities will be required for guideline compliance. It is our assumption that the proposed “Accountable Care Organizations” that are the backbone of the recent US health insurance reform legislation reflect this understanding in a larger context, and are an effort to place this understanding into practice outside of Kaiser Permanente.
CIN2: TO TREAT OR NOT? HOW DO I SAY NO?

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Objectives: Simply put, the management of CIN is dichotomous. Yet the histologic classification of CIN still has more than 2 categories. In most clinical settings, CIN1 is considered the histologic manifestation of what is usually a transient HPV infection and hence is worthy of conservative management. CIN3 is uniformly agreed upon as a pre-cancerous lesion that must be ablated to prevent cancer. What about CIN2? To argue that CIN2 should not be treated, like CIN1, implies that CIN2 is a distinct pathology that biologically behaves like CIN1. Yet, there is still considerable controversy on this point as in the U.S. and at least parts of Europe, a diagnosis of CIN2 + is the clinical threshold for ablative therapy. Indeed, some actually think the opposite, i.e. CIN2 is more like CIN3. Can it be both? Can we potentially segregate the good CIN2 from the bad?

Methods: Data on the interpretive variation of CIN diagnoses, the age distribution of CIN grades, the HPV types found in CIN2 vs CIN1 and CIN3 and biomarker correlates are presented.

Results and Conclusions: The histologic diagnosis of CIN2 is poorly reproducible compared to CIN1 and CIN 3. Women with CIN2 are neither younger than women with CIN3, nor is CIN2 a more common diagnosis. In addition, the majority of women with a routine CIN2 diagnosis on biopsy are not subsequently diagnosed as CIN2 based on the review of a subsequent LEEP. In contrast, adjudicated or consensus diagnoses of CIN2 are much more likely to have a subsequent LEEP diagnosed as ?CIN2 or ?CIN3 and this outcome is strongly correlated with the presence of HPV16, high grade cytology and high grade colposcopic impression and p16 positivity. Thus, CIN2 diagnoses represent an equivocal diagnosis, much like ASC-US cytology. In our opinion, the bulk of the evidence strongly suggests that CIN2 is not a real disease state, but a misclassification of biologic CIN3 or CIN1. For the subset that may represent CIN1, there may be the potential for conservative management, and p16 may be a useful biomarker in this regard.

MOVING FROM ALGORITHMS TO RISK BASED MANAGEMENT: IS IT OF VALUE FOR THE CLINICIAN? YES

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Advances in vaccination, screening, and diagnosis make it increasingly possible to prevent cervical cancer. However, if misused or poorly understood, these new tools will only increase costs and potentially harm patients without benefit. As a framework for standardized care that maximizes patient safety and wellbeing, a risk-based approach should be adopted to guide clinical management now and in the future. The model would use thresholds of increasing risk for cervical precancer and treatable cancer to guide clinical decision-making for screening intensity, diagnostic evaluation, or treatment. For example, carcinogenic human papillomavirus (HPV)-negative women are at very low risk of cervical precancer and cancer (<1%) over the next 5-10 years and can be screened less frequently, i.e., longer intervals between screens. Extending screening intervals among carcinogenic HPV negative women is necessary for preventing over-diagnosis and treatment since newly-detected HPV infections that convey much lower risks of concurrent and future cervical precancer and cancer than prevalently-detected carcinogenic HPV infections of unknown duration. Such a model would help integrate screening and HPV vaccination because HPV vaccination will variably reduce the risk of cervical precancer and cancer depending on the age of vaccination (relative to the age of sexual debut). More generally, the meaning, or the positive predictive value, of a positive screening result will differ based on past history such as HPV vaccination and screening history. A group of us are now working on developing a risk calculator to integrate past and current risk stratifiers/modifiers to provide risk estimation in a user-friendly manner. Importantly, experts would establish risk thresholds and stratum for clinical actions based on the patient risk to benefit, independent of current (e.g., cytology, carcinogenic human papillomavirus testing, and colposcopy) and future methods of measuring risk (e.g., HPV genotyping and p16 immunocytochemistry). Decisions regarding clinical management would be based on individualized risk measurement rather than increasingly complicated algorithms that are often difficult to understand and poorly adopted. Using a risk-based management model for cervical cancer prevention, based on appropriate clinical actions that correspond to risk stratum, can result in better allocation of resources to and increased safety for women at the greatest risk and increased well-being for women at the lowest risk.
A number of new assays have recently been introduced for HPV testing. Here we review the characteristics of different assays and highlight their strengths and weaknesses. Direct comparisons between tests in the PREDICTORs studies will also be reviewed.

We reviewed two studies in which 7 physicians performed 1,383 colposcopic examinations on women with cervical cytology of cancer, HSIL, LSIL, or ASC-US with positive HR-HPV to estimate the increase in yield of cervical intraepithelial neoplasia (CIN) 3 or cancer (CIN 3+) per colposcopy when ‘random’ biopsies in cervical quadrants without visible lesions and endocervical curettage (ECC) were performed in addition to colposcopically directed biopsy. [Pretorius RG et. al. JLGTD;2011] These 1,383 women were drawn from 10,425 women without missing data that participated in two cross-sectional comparative trials of techniques to detect CIN performed in Shanxi Province, China between June 1999 and April 2001. [Belinson JL, et. al. Gynecol Oncol;2001 and Belinson JL, et. al. Int J Gyn Cancer;2003] In these trials, at colposcopy, the cervix was divided into quadrants by lines from 12 to 6 and 3 to 9 o’clock. Each cervical quadrant had a colposcopic impression. Colposcopically detected lesions were biopsied. Cervical quadrants with normal colposcopic impressions had ‘random’ biopsies at the squamocolumnar junction. Lastly, ECC was obtained.

The mean age of the 1,383 women was 40.8 years (32-50 years). 141 of 222 (63.5%) women with CIN 3+ were diagnosed by biopsy of a cervical quadrant with a colposcopic impression of human papillomavirus, CIN, or cancer (colposcopically directed biopsy); 57 (25.7%) were diagnosed by a ‘random’ biopsy in a cervical quadrant with a normal colposcopic impression; and 24 (10.8%) were diagnosed solely by a positive ECC. The yield of CIN 3+ per colposcopically directed biopsy (240/1,205, 19.9%) was 7.8 times higher than that of ‘random’ biopsy (109/4,304, 2.5%, p<.001). As shown in figure below the increase in yield of CIN 3+ per colposcopy associated with up to four ‘random’ biopsies plus ECC in addition to biopsy of cervical quadrants with colposcopic impressions of HPV, CIN, or cancer was significant for all doctors (p=.03 to p<.001) except for doctor #5. The proportion of CIN 3+ involving 3 or 4 cervical quadrants for doctor #5 (50%, 7/14) was over three times that of the mean proportion of CIN 3+ involving 3 or 4 quadrants for the other six doctors (15.4%, 32/208, p=.001).

We conclude that biopsies in cervical quadrants without visible lesions and ECC in addition to colposcopically directed biopsy will increase the yield and decrease the risk of missing CIN 3+. 
THE BEST MANAGEMENT OF HPV POSITIVE WOMEN

G. Ronco & A. Antila

Outline

- In the recent trials on primary HPV screening, there have been various ways how HPV test has been integrated with the on-going screening programme.

- Based on comparisons on the predictive values (PPV) among women with a positive HPV test, cytology triage is recommended.

- Protocols have varied, however, considering what to do when the HPV test has been positive and the cytological result normal.

- HPV testing should be avoided, however, among women younger than 35 years, due to high probability of women with a positive HPV test.

- P16 is an example of potential new biomarkers that could be considered. More research on which would be optimal biomarkers is needed.

VERIFICATION BIAS: DOES IT MATTER? YES

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Verification (or work-up) bias is defined as an error in the estimation of the sensitivity and specificity of a screening test when the extent of disease verification differs according to test result. In other words, the bias occurs when the frequencies of individuals in the cells of the core 2 by 2 table (true positives, false positives, false negatives, and true negatives) that is used to derive sensitivity and specificity are not representative of the true distribution of test results by disease status. For a rare disease, the bias will result in an overestimation of the sensitivity and underestimation of the specificity, thus leading to mistaken assumptions about the performance of a screening test if applied as a population intervention. Verification bias can be circumvented in the design of the study if a provision is made to verify the presence of disease in a sample of or among all individuals whose test results are negative. Frequently, subjecting test-negative individuals to a diagnostic procedure is not a realistic proposition on ethical or logistic grounds, which has led to much debate about the practicality of the approach. Statistical methods are available to correct the sensitivity and specificity estimates based on the disease rate in the test-negative sample. In the field of cervical cancer screening there are several examples of studies that controlled for the bias. The author will present examples of how the bias occurs and discuss the advantages and disadvantages of controlling for it.
CS 2-6

VERIFICATION BIAS: DOES IT MATTER? NO

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Verification bias has several forms. One that does need to be adjusted for is non-compliance to colposcopy/biopsy in women with abnormal screening results. However it has also been suggested that verification bias should also be adjusted for to account for disease in women who are negative on (all) screening tests. Although theoretically desirable unless a high proportion of such women are sent for colposcopy/biopsy (which many consider to be unethical), this is a highly unstable process. It has the unfortunate propriety of replacing a reliable estimate of relative sensitivities, etc, with unreliable estimates of absolute sensitivities, and this adjustment should be avoided in most circumstances. Some hypothetical examples of its disastrous effect will be discussed.

216

CS 2-7

ADVERSE PREGNANCY OUTCOMES ASSOCIATED WITH EXCISIONAL TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA: AN UPDATED META-ANALYSIS

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Objective: To update prior meta-analyses assessing the relative risk of adverse pregnancy outcomes associated with excisional treatment of cervical cancer precursors.

Material and methods: Medline and Embase citations were tracked from January 1960 to December 2010. Eligible studies were selected if data on adverse pregnancy outcomes were provided for women with and without prior treatment with LLETZ (large loop excision of the transformation zone) or CKC (cold knife conisation).

Results: One prospective cohort and 31 retrospective studies were retrieved. LLETZ was associated with an increased risk of preterm delivery [PD] (<37 weeks: RR = 1.67; 95% CI: 1.41-1.97) and low birth weight [LBW] (<2500gr: RR = 1.74; CI = 1.43-2.12). However, no statistically significantly increased risks were identified neither for extreme PD (<28 weeks: RR = 1.56; CI: 0.57-4.30); nor for severe low birth weight (<2000gr: RR = 1.29; CI: 0.43-4.00), nor for peri-natal mortality (RR = 1.26; CI: 0.97-1.82). Statistically significant associations were noted for CKC regarding PD, LBW but also regarding severe PD and extreme LBW.

The risk of PD increased significantly with the height of excised tissue (CKC or LLETZ).

Most often control women were selected from the general population not suffering from CIN. Therefore, additional subgroup meta-analyses were performed restricting the control groups to non-treated women with cervical pathology or women with pregnancies before diagnosis or treatment of CIN. In these subgroup meta-analyses, LLETZ was not associated any more with PD (RR = 0.78; CI: 0.51-1.48) or LBW (RR = 1.00; CI: 0.56-1.80). However, after CKC, the risk of PD and LBW still was significantly increased (RR = 2.87; CI: 2.24-3.69).

Discussion: Women treated with LLETZ are at increased risk of adverse obstetrical outcomes but not of severe adverse outcomes. At least a part of preterm and low-birth weight deliveries may be due to factors associated with CIN.

Women treated by CKC are at increased risk of moderate and severe adverse outcomes. At least a part of this increased risk can be attributed to the treatment procedure.
TREATMENT OF CIN DOES NOT CAUSES PREMATURE DELIVERY

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Preterm delivery remains a significant cause of perinatal morbidity and mortality but there is no single underlying cause. Worldwide data is limited but the rate of preterm delivery is estimated to range from 5% in developed countries to 25% in developing countries. The preterm delivery rate has been relatively stable around 5-10% in developed countries for many years, despite changes in colposcopy practice, with planned delivery accounting for about 50% of cases. Factors associated with spontaneous preterm birth are multiple pregnancy, previous preterm birth, young maternal age, BMI <19, low socio-economic status and cigarette smoking. Cervical incompetence is a rare cause of preterm labour but we cannot ignore the evidence which suggesting that treatment of CIN causes preterm delivery.

The data available can be difficult to interpret and we need to remember that the correlation between preterm delivery and treatment of CIN does not automatically imply that one causes the other. Larger studies have already identified that at least part of the increased relative risk of preterm delivery relates to common causal factors.

Both cervical stenosis and incompetence are well recognized complications of cold knife cone (CKC) biopsy. To conserve reproductive function, treatments of CIN, CGIN and micro invasive cervical cancer have moved to less radical excisional treatments and for CIN per se to excisional or ablative treatment. Since we believe that high grade CIN is a precursor of cervical cancer, we need to be certain that we pose the correct clinical question and take care to evaluate the evidence. For example, the inclusion of cases treated by radical treatments for CGIN and cancer are not relevant to the treatment of CIN. Treatment of CIN necessitates removal of the transformation zone to a depth of 7-8mm either by ablation or excision. There is no clear hypothesis on how the higher temperatures involved in cold coagulation to produce thermal coagulation effects are less disruptive to cervical function than the lower temperatures of radiofrequency electric current to cut tissue or achieve haemostasis in LLETZ or LEEP. It is more plausible, that excessive depth of treatment (a known risk factor) is responsible. Current evidence is limited as the amount of cervical tissue removed is not available in large studies of routinely collected data. If cervical damage is a recognized complication then we need to take the same approach to any surgical complication and improve our training and practice of the treatment at colposcopy if we believe that it is an effective means of reducing the risk of cervical cancer.

ANAL CANCER: TO SCREEN OR NOT TO SCREEN

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Background: If anal cancer rates are rising, especially in men who have sex with men and immune compromised individuals (particularly those with HIV infection) are at highest risk and high-grade anal intraepithelial neoplasia is the precursor, should at risk individuals be screened? The goal is to find early HGAIN and ablate it to prevent progression to invasive anal squamous cell carcinoma (ASCC). No prospective randomized trials have been carried out to date, but many clinicians believe that, as in the cervix, treatment of dysplasia will ultimately prevent cancer.

Discussion: Rates of progression of HGAIN to anal cancer are probably lower than rates of progression in the cervix. However, Bowen’s disease has identical histology to HGAIN and clinicians agree that it is an ASCC precursor and should be excised. Moreover, published rates of progression from series watching HGAIN for progression have shown progression to AASCC from 3% to 11% and it can occur rapidly. In contrast treatment studies with long term follow-up have demonstrated progression rates below approximately 1%. ASCC treatment, while most often curative can result in significant morbidity. Treatment of HGAIN has high recurrence rates but that is most often due to developing metachronous lesions. Cure rates of individual lesions remain high. There is little morbidity to treating HGAIN. Targeted ablation of CIN in HIV infected women also has very high recurrence rates. Moreover, would a randomized controlled trial of treatment vs. observation be ethical with this evidence?

Conclusion: Retrospective series on treatment of HGAIN have lower rates of progression to ASCC than series of expectant management. Treatment of HGAIN has lower morbidity than wide local excision or chemo/radiation therapy. Treating HGAIN probably prevents progression to ASCC.
HPV VACCINE FOR HIGH RISK POPULATIONS: IS IT OF PUBLIC HEALTH VALUE? - YES

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Although nearly all countries have now adopted healthcare policy statements recommending HPV vaccination, different countries are using different systems for the introduction of immunisation. Some countries have adopted school-based systems whereby all girls are routinely recommended and offered free vaccination at age 12/13 years, whereas other countries are using healthcare provider funded systems based on local policy, physician recommendation, and/or patient request. Another level of complexity has been added by the demonstration of efficacy of the quadrivalent HPV vaccine in prevention of HPV disease in men, the endorsement of this use by the FDA, and the current consideration of such by the EMA.

Mathematical modelling has provided key insights into the transmission dynamics of sexually transmitted infections, and has lately provided further insights into the predicted medium- and long term effects of HPV vaccination at the population level. I will present data to support the utility of HPV vaccination in high risk populations –

(a) As part of population based HPV vaccine programmes
(b) As targeting core groups and high frequency HPV transmitters
(c) As male vaccination in the setting of population based female vaccination to contribute towards HPV disease eradication

HPV prophylactic vaccines as primary prevention measures are expensive compared to other preventive measures such as the older vaccines. But their value expressed as quality adjusted life year (QALY) is very cheap in the HPV naive population compared to other preventive measures. QALY of HPV prophylactic vaccine in low HPV prevalence population is cheaper than in HPV high prevalence population. HPV prophylactic vaccines have to compete with other new vaccines such as shingles, pneumococcal conjugate vaccine or rotavirus vaccine for budget. So benefits may be seen with the use of HPV prophylactic vaccine in some high populations but their public health value may be much lower than other preventive measures. If HPV prophylactic vaccines would be cheap, it would not be difficult to allocate resources to have HPV vaccines for all since the QALY would be even cheaper. But in a competing market, HPV vaccines may not be the best allocation of rarer budgetary money for high risk population. Most of the high risk population such as commercial sex workers of both sexes, injection drug users and men having sex with men may have already been exposed to most of the HPV vaccine types making the value of the QALY too expensive to qualify for budget allocation on a public health program basis. Exception might be those patients with HIV infection acquired through maternal child transmission or blood borne origin with minimal number of partners. Preventive measures such as STI screening and counseling might be better use of limited preventive money in high risk population. School based and pre sexual debut vaccine program may represent the best use of limited preventive budget in HPV prophylactic vaccine program. Cervical cancer screening program may be allocated better resources to reach for high risk women.
While the incidence of any particular HPV related cancer in men is low, the total cancer burden related to HPV in men is quite high, and similar to that experienced by women in countries that routinely screen for cervical cancer. Unlike the majority of HPV related cancers in women, there are no proven screening measures to prevent HPV related cancers in men. Of particular concern is the fact that two cancers caused by HPV in men are significantly increasing each year – HPV related oropharyngeal and anal cancers. Both cancers result in tremendous loss in quality of life both during and after treatment. Both cancers are costly to treat, and both cancers ultimately result in mortality. Prevention of the HPV infections that cause cancers in men is the only viable option to reduce HPV related cancers in men.

In addition to a direct benefit males can receive from HPV vaccination, there is the indirect benefit females will receive if males are vaccinated. Reductions in male HPV infection will ultimately lead to reduction in HPV infection and disease in females. This is especially true for countries such as the US where less than 50% of females completed the three dose sequence (currently 23% in the US). In most countries where there is not mandatory HPV vaccination it is doubtful that 75% of females will complete the three dose series of HPV vaccine in the coming decades. With a low uptake of vaccine in females, male vaccination becomes an important part of establishing herd immunity and reducing infection and disease caused by HPV in females, and under this scenario is cost-effective.

To achieve public health benefit and opportunity the HPV vaccine presents, both sexes should be vaccinated. Men should have the opportunity to directly benefit from the newly licensed vaccine for boys.
Whether HPV vaccination is to be recommended for adult women depends on:

1. Licensure
2. Secondary prevention options
3. Existing HPV vaccination programme (if any)
4. Individual risk assessment
5. Health insurance (preventive vs. curative)

I will argue that reimbursement should be considered under specific conditions.

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**SHOULD THE HPV VACCINE BE USED FOR PRIMARY PREVENTION OF CERVICAL CANCER AMONG ADULT WOMEN? THE “NO” SIDE OF THE DEBATE**

**LAWSON H:**

*department of gynecology and obstetrics, Emory University school of medicine, Atlanta, GA, US.*

**Objectives:** Demonstrate that currently available HPV vaccine use not be recommended for preventing cervical cancer and its precursor lesions among “adult women.”

**Methods:** Define adult women for purposes of receiving HPV vaccine. Describe evidence from clinical trials and results of cost effective analyses to show suitability of using currently licensed preventive bivalent and quadrivalent HPV vaccines among “adult” women.

**Results and Conclusions:** Results of clinical trials and cost effectiveness analyses will demonstrate that periodic screening and follow up of adult women, most of whom already have been exposed to the high risk HPV types, is more effective for preventing adverse outcomes of HPV infections--progression to high grade CIN and invasive cervical cancer--than the receipt of a full series of the currently available HPV vaccines. Therefore, currently available bivalent or quadrivalent HPV vaccine use should not be recommended for use among women beyond the age limitation currently suggested by existing evidence and organizational recommendations.
Why is immunogenicity testing important? Protection induced by prophylactic vaccines is largely, if not entirely, mediated by antibodies that prevent virus infection. In licensure it provides key elements:
1) quality control relating to vaccine manufacture, stability in storage and distribution
2) Seroconversion rates, antibody levels and longevity which might predict duration of protection
3) Bridging studies to link efficacy trials to wider and specifically younger women.
4) Assessment of the relative merits of different vaccines in absence of comparative efficacy trials. There is no precise knowledge of any immune correlate of protection in natural HPV infection although only about half of women exposed to oncogenic HPV infection make antibodies to the viral capsids and the low titres induced are not necessarily sufficient to prevent a subsequent infection. There is currently no serological assay that can accurately mimic real HPV infection as a means to assess either natural or vaccine induced HPV specific antibody responses. All available assays ELISA, Competitive Luminex, Pseudo-Neutralisation or Cervicovaginal Murine Challenge models are surrogates which may or may not encompass the complete range of antibody specificity, affinity or titre relevant in prevention of natural infection in the anogenital tract. The virtually 100% seroconversion rates associated with HPV VLP vaccination and superiority over natural levels for at least five years complicates defining a serological correlate of protection. Immunogenicity data can be useful as predictor of efficacy if a lower level correlates with either breakthrough of HPV infection or a higher value with additional (cross) protection. Looking for breakthrough in vaccinated populations requires sufficient follow up of clinical trials where the attack rate of viral infection would be sufficient to conclude the threat was still controlled. Differences in cross protection against non-vaccines types which correlate with higher titres of HPV 16 and 18 antibodies can broadly support a relationship with efficacy. These conclusions need to be supported by continued follow up.

Debate points are:
- Uptake of vaccination programmes.
- School-based versus non-school based programmes.
- Enhancing herd immunity effects.
- Equity.
- Sexual activity and age.
- Allocation of health care budgets over a longer period.
First Years of Implementation of HPV Vaccination in the Benelux

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Objective: To report on the introduction of HPV vaccination in three closely related European countries: Belgium (BE), the Netherlands (NL) and Luxembourg (LX).

Methods: Data were collected from the National Institute for Health and Disability Insurance and the Inter-mutualistic Agency (BE), the National Institute of Public Health and Environment (NL), and from the Ministry of Health and the ‘Caisse nationale de Santé (LX). Sales statistics were obtained from the Intercontinental Marketing Services (IMS Health).

Results: Advisory boards in all three countries advised organized HPV vaccination of girls of 12 years with variable catch-up policies. In BE, the national health authority partially reimburses the bi- and quadrivalent HPV vaccines (initially for girls of 12-15 years, later extended until 18 years). School-based free vaccination for a single cohort (~12 years of age) with the quadrivalent vaccine was set up in the Flemish Community in 2010, but not in the other Communities. In NL, universal HPV vaccination for 12 year old girls was integrated in the national vaccination programme in 2010, using the bivalent HPV vaccine. This was complemented with a short term catch-up programme for girls born between 1993 and 1996. In LX, 12-year-old girls are invited for free vaccination with the bi- or quadrivalent vaccine since 2008, but the HPV vaccine is also free of charge for female adolescents of 13-17 years.

Mid-2009, in BE, the 3rd dose coverage built up through the partially reimbursed opportunistic vaccination decreased progressively by cohort: from 41% (cohort 1992) to 14% (cohort 1995). End 2010, in NL, the 3rd dose vaccine coverage dose was 52% for both the catch-up and the national programme. In LX, by the end of 2010, 11,774 girls had received at least one dose of HPV vaccine and 79% of them had completed the schedule of 3 doses (~40% of girls passing through the target age group in 2008-10). Sales statistics from BE and LX indicate a decreasing trend in the consumption of HPV vaccines since 2009.

Conclusion: Fully government funded routine HPV vaccination, set up only in the NL, resulted in higher vaccine uptake than opportunistic or partially funded approaches (BE, LX). Moreover, in contrast with other vaccines in their routine programme, HPV vaccine uptake in BE and LX decreases over time. Fully funded, preferentially school-based, programmes should be set up in all three countries.

Basic HPV Immunology

Stern P.

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The oncogenic HPV lifecycle is characterised by infection in basal cells of anogenital epithelia with virus production dependent on epithelial differentiation and virions produced only in terminally differentiated cells. Natural control of an oncogenic type HPV infection depends on appropriate activation of innate immune mechanisms leading to stimulation of adaptive immunity in the form of specific T cells against viral proteins such as E2, E6 and E7 which can effect clearance of the virally infected cells. Neutralising antibodies are detected in about 50% of women who have cleared such infections but are not necessarily protective against future infection by the same HPV type and are never therapeutic. In addition, the stealthy lifecycle of the virus which causes little of no damage and no viraemia as well as various viral immune evasion strategies sometimes allows the virus to avoid immune detection providing for persistent infection which is the major risk factor for development of intraepithelial neoplasia. Oncogenic HPV infection is necessary but insufficient for development of a cervical cancer and additional genetic changes are acquired over time through selection including those that allow for immune escape from immune surveillance.
DETECTING AND ASSESSING ADVERSE EVENTS FOLLOWING IMMUNISATION: HOW TO DISTINGUISH CAUSE AND COINCIDENCE

Lopalco PL.
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Immunisation activities are often conducted as large scale campaigns involving high proportions of the population. It is very likely that some unfavourable events occur few days after the administration of a vaccine. This is particularly true during the childhood when the vast majority of infants and children undertake repeated immunisation visits in a short timeframe. Those events are classified as Adverse Events Following Immunisation (AEFI) and their detection is pretty well regulated at European level. When reported, AEFI should be carefully assessed in order to either accept or exclude a real link between vaccine administration and adverse event. Such assessment should be the result of a careful clinical assessment (at individual level) together with epidemiological and statistical analysis (at population level). Many new methodological tools have been developed during the recent years in order to distinguish real cause and coincidence. In particular, the self-controlled case studies are shown to be very useful in this field. The availability of large databases that might be cross-linked at population level would also improve both the ability to detect rare AEFI and to assess the causality relationship.

HOW TO IMPROVE PARTICIPATION

Brotherton JML
National HPV Vaccination Program Register and Victorian Cervical Cytology Registry
Victorian Cytology Service
East Melbourne, Australia

In this presentation I will review current knowledge about factors that are influential in determining HPV vaccination coverage and how we might address barriers to achieving high vaccine coverage. Consideration will be given to:

- Lessons learnt from other vaccination programs
- Emerging experience with HPV vaccines in particular
- The three dose issue
- Why accurate coverage data is important
- Why equity matters
**SPC 1-7**

**SOCIAL, ETHICAL AND COMMUNICATION ISSUES**

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*President of Univers'Elles, a women’s association and MSD, Brussels, Belgium*

**Objectives:** An analysis to assess the role and impact of communication in the introduction and implementation of vaccination programs. The analysis also looks at future perspectives for vaccination advocacy, taking into account social and ethical issues.

**Methods:** Analysis of processes and trends unfolding in Europe specifically and literature research.

**Conclusions:** In January 2011, the WHO chief suggested that perceptions around vaccination had been irreversibly damaged by a series of scare stories, “to an extent that no amount of evidence can change the public’s mind.” As cognitive relativism reigns nowadays, the boundaries between facts and beliefs have become increasingly porous. Facts only are not enough to do the job and it thus appears high time to rethink the approach to communication to become more transparent and responsive to public concerns. First, it is becoming clear that it is only through concerted and active efforts of all stakeholders, including policymakers, health care professionals, industry and civil society that faith in vaccination can be restored. Second, these stakeholders need to engage the public because people trust each other first. New communication channels, especially social media, have to be part of this engagement strategy. Third, public health communication campaigns should now go beyond public education to include public persuasion. A number of approaches can be leveraged from social marketing, peer education to nudging. These approaches will be reviewed and debated, since each has a distinct social and ethical impact.

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**STC 1-1**

**VULVAR PAIN**

Jacob Bornstein M.D., MPA,  
*President Elect, the International Society for the Study of Vulvovaginal Disease (ISSVD)*

*Professor and Chairman, Department of Obstetrics and Gynecology, Western Galilee Hospital and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Nahariya, Israel*

A common cause of vulvar pain, especially dyspareunia, is localized provoked vulvodynia (LPV). An operative procedure called perineoplasty was advocated in 1981 by JD Woodruff for the treatment of severe LPV. It included vestibulectomy and vaginal advancement and has been used successfully since then. Several non-surgical treatments that have also been proposed. Recently, placebo-controlled studies have proved that Desipramine oral treatment, Lidocaine topical cream and Nifedipine topical creams are no better than placebo in relieving LPV. Our finding of increased tissue heparanse and epithelial hyper innervation are the basis for anti-heparanase treatment. Difficult cases include: combined provoked and unprovoked vulvodynia, and association of LPV with other local or systemic conditions.

**Table: Evaluation of a new patient with vulvar pain**

1. History, including localization of the pain (entry or deep), provoked or unprovoked pain  
2. Determine the presence of sensitivity to urination, tampon insertion, and level of dyspareunia  
3. Assess previous treatments  
4. Examination: exclusion of other causes of dyspareunia, Q-tip test  
5. Providing information, reassurance and treatment plan  
6. Referral to a support group  
7. Consider: consultation with a psychiatrist or with a sex therapist in selected cases
Objective: To discuss epidemiology, diagnosis, treatment and preventin of vulvar intraepithelial neoplasia (VIN)

Methods: Review of available data

Conclusions: An increasing incidence of VIN in young women was observed. This was followed by an increasing incidence of HPV related vulvar cancer in younger women. Low grade VIN is mainly caused by HPV 6, high grade VIN mainly by HPV 16, 31 and 6. Data from the quadrivalent HPV vaccine have demonstrated a 100% prevention of VIN caused by the four vaccine types. Diagnoses has to be histology based, abnormalities at the vulva should be biopsied. However biopsies may miss an invasive process. Treatment of choice is still surgery, it has the advantage of full histopathological evaluation. Laser surgery may be applied after mapping, but should be limited to experienced centers. Imiquimod has been shown to be an effective topical treatment. Therapeutic vaccines are under clinical evaluation.

RESULTS OF HPV TYPING IN WOMEN OF A REPRODUCTIVE AGE

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FGU Scientific Center of Obstetric, Gynecology and Perinatology named after V.I. Kulakov, Moscow, Russia

Objectives: Revealing of oncogen genotypes of human papillomavirus (HPV) is considered to be an important stage of the diagnosis of cervical intraepithelial neoplasia (CIN) in women of a reproductive age. It is well known that spectrum of HPV genotypes differ in different regions of the world.

Methods: In order to identify the most frequently encountered HPV genotypes in cervical cell samples from 350 HPV/DNA positive patients from Russia were evaluated by PCR. As a result 21 HPV types were identified and ranged according to their frequency: HPV 16 – 19.0%, HPV 58 – 9.2%, HPV 52 – 7.8%, HPV 31 – 6.6%, HPV 68 – 5.5%, HPV 39 – 5.4%, HPV 56 – 5.1%, HPV 6 – 4.7%, HPV 45 – 4.6%, HPV 53 – 4.4%, HPV 44 – 4.1%, HPV 33 – 3.7%, HPV 66 – 3.2%, HPV 18 – 2.9%, HPV 35 – 2.6%, HPV 59 – 2.3%, HPV 73 – 2.1%, HPV 82 – 1.4%, HPV 26 – 0.6%, HPV 11 – 0.2%. The average rate of the HPV types per sample was 1.87. There were more than 5 different HPV types in 10 samples (2.9%).

Conclusion: The results suggest that the HPV 16, 58 and 52 are the most frequently encountered HPV genotypes in our study. Our results differ from literature data commonly mentioning the HPV 16, 18, 31, 33 as the most frequently encountered HPV genotypes in European women.
**EW 2**

**FIGHTING CERVICAL CANCER IN RURAL INDIA: BUILDING A GRASSROOTS MODEL WITH COMMUNITY HEALTH WORKERS**

Krishnan, S,
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**Objectives:** India has the highest number of cervical cancer cases in the world. One woman dies of cervical cancer every 7-8 minutes in India each day. Yet, this is the only cancer that is almost completely preventable by safe, simple, and inexpensive methods. Lack of an organized system for screening is the main reason for these high rates. In addition, there are severe shortages of qualified human resources for health. Hence innovative methods to control this disease in a scientific and culturally sensitive manner are needed. This situation has triggered an interest to shift less demanding tasks from highly trained health workers to less highly trained health workers through a process of task shifting that is based on the principle of optimization. This project is designed for capacity building. Numerical results, pros and cons of the current model, and challenges and opportunities encountered in this low resource setting will be discussed.

**Method:** In a remote district in India, a grassroots effort is being developed between trained Dais (midwives) from an NGO, who raise cervical cancer awareness in the community, and a local hospital that provides mass screening. Twelve health workers were sent for two weeks training in cervical cancer screening. As “one stop screen-and-treat program” is not available, gynecological camps staffed by physicians are held every two months to treat women who test positive and appropriate treatment/ referral instituted. 421 women have undergone screening so far. 73 tested positive for VIA. 65 returned for follow up. 29 required biopsies. 5 underwent cryotherapy. No invasive cancer was detected in this group.

**Conclusion:** As only 40%-60% of the total number of eligible women recruited turned up, there needs be more creative ways to motivate and empower women in the community. Better incentives for dais, getting men involved (in male dominated societies), and community ambassadors will be essential to improve patient participation. Not all women who tested positive for VIA returned for follow up showing “one stop screen and treat program” will have a better outcome as evidenced by a large body of international studies. For future scaling up of the project, specific criteria should be applied while selecting community health workers to ensure full participation in the program. Due to lack of an organized system for cervical cancer screening, India would benefit greatly from the prophylactic HPV vaccines.

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**EW 3**

**HPV VACCINATION IN CZECH REPUBLIC**

Fait T.

Dept. of Obstetrics and Gynecology, General Faculty Hospital Prague, Charles University Prague, Czech republic

**Objectives:** The aim of study was to map the HPV vaccination choices in population of Czech republic.

**Methods:** In first part of study total amount of vaccinated women was counted. The second part has evaluated age distribution of vaccinated women in sample of them.

**Conclusions:** The possibility of HPV vaccination started in December 2006 in Czech republic. On the end of year 2010 totally 145 000 women were vaccinated, it means that around 12% of women in age 9 – 26 years are cover by it. In common practice about 6% of vaccinated women are over 26 years and 38% in age 15-17. The proportionality of quadrivalent and bivalent vaccine is 71% / 29%. 
DEVELOPING RESOURCES TO SUPPORT PRIMARY CARE HEALTH PROFESSIONALS IN CERVICAL SCREENING AND HPV: PRELIMINARY RESULTS FROM ATHENS

McSherry L1, Dombrowski S2, Francis J3, Murphy J1, Martin C4, O’Leary J4, Sharp L1 for the ATHENS Group

1 National Cancer Registry, Cork, Ireland; 2 Newcastle University England; 3 University of Aberdeen, Scotland 4 Coombe Women’s Hospital, Dublin, Ireland

Objectives: In Ireland, as elsewhere, primary care health professionals play a key role in cervical cancer prevention. As well as providing services via a national screening programme, they provide advice about human papillomavirus (HPV) infection, testing and vaccination. Thus, their knowledge and practices in these areas will have a major influence on the success of cervical cancer prevention in Ireland. The aims of ATHENS (A Trial of HPV Education and Support) are to (1) develop practical resource(s) to support health professionals in this area; and (2) test the efficacy of these resources in improving knowledge and influencing clinical practice.

Methods: The resource(s) will be developed through primary research based on theories of behavioural change. In-depth semi-structured telephone interviews and a quantitative survey will be conducted amongst primary care doctors and nurses. This will identify the (1) key clinical behaviours/practices that the resources will seek to influence; (2) frequency and distribution of key behaviours; and (3) modifiable determinants which predict key behaviours. A randomised controlled trial will be undertaken to test the efficacy of the resource(s). Strategic and logistic input is provided by key stakeholders and user groups. So far, interviews have been conducted with 12 doctors and 14 nurses. Preliminary analysis has revealed that male doctors are moving away from the role of smear taker. HPV infection is not widely discussed with patients. Reasons cited include their own lack of knowledge, avoidance of the topic, unwillingness to embarrass patients, and uncertainty about how to tackle the subject. Cost emerged as a major barrier to conducting HPV vaccination in general practice. Professionals’ awareness of HPV testing is limited and there is considerable uncertainty regarding its clinical benefit and role in management. Additional interviews will be conducted and results presented for all interviews.

Conclusions: As well as supporting primary care health professionals in Ireland, ATHENS will help to ensure that women receive the most up to date information and appropriate advice regarding cervical cancer prevention.

MEN AND HPV: WHAT THEY KNOW AND WHAT THEY NEED TO KNOW

Pitts MK

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Objectives: There is increasing recognition that HPV is implicated in a range of cancers, not all of which are solely associated with women. Most vaccination programs are targeted at young women and marketed as preventing cervical cancer only. This presentation reviews recent national data on the knowledge, attitudes and beliefs about HPV amongst Australian men and women to gauge the need for a more inclusive approach to health messages concerning HPV, vaccination and associated health risks, especially for non-genital cancers.

Methods: The study draws on two samples, one is a nationally representative sample of Australian young men and women aged 16-18 years (N= 2,924), and the second is a nationally representative sample of Australian men and women between the ages of 18 and 64 years (N=8,650). Both samples were recruited in late 2008 and early 2009. Both studies indicate marked and highly significant gender differences in knowledge, attitudes and behaviours relevant to HPV and the HPV vaccination.

In the adolescent study, 12% of young men compared with 29% of young women believed that HPV affects only men, while 21% of young men compared with 39% of young women believed it affects both men and women. Nine per cent of young men compared with 17% of young women knew of the association of HPV with genital warts; in contrast, only 15% of young men compared with 46% of young women knew of the association with cervical cancer.

In the adult survey, 50% of men had heard of HPV compared with 73% of women. Adult participants were asked: Do you know of any health problems caused by this virus – HPV ? Analyses of the 1,278 responses showed marked differences between men and women in both accuracy and range of answers, with men more likely to name cancers other than cervical cancer, and with clear confusion for both men and women in the range of health problems thought to be associated with HPV. Only 20% of men, compared with 82% of women, spontaneously named cervical cancer as a health problem caused by HPV. Men and women were equally concerned about fertility risks they believed to be associated with HPV.

Conclusions: There is a clear need to include men in public education programmes concerning HPV and to address the gendered approach to HPV currently adopted by most governments and health systems.
EWWWEN'S VIEWS OF HPV VACCINATION IN IRELAND: FINDINGS FROM A NATIONAL POPULATION SURVEY
O’Connor M, Murphy JC, Sharp L, on behalf of the Irish Cervical Screening Research Consortium (CERVIVA)
National Cancer Registry Ireland, Cork, Ireland

Objectives: A school-based HPV vaccination programme, based on administration of Gardasil® to girls aged 12-13 years, was rolled-out across Ireland in late 2010. Primary care practices are promoting HPV vaccination for older girls and women. Women’s views regarding vaccination are likely to influence uptake. We undertook a national population-based survey to determine knowledge of, and views towards, HPV vaccination among women in Ireland.

Methods: An age and area-stratified random sample of women aged 20-64 was selected from primary care practices and Well Woman centres across Ireland. A questionnaire was developed from literature review and focus groups and included questions on cervical screening, HPV infection, HPV testing and HPV vaccination. This was mailed to 5,553 women during August and September 2010.

Conclusions: 3,345 completed questionnaires were received (response rate=60%). This analysis includes the first 1,654 respondents. 55% of women had heard of a vaccine against HPV prior to completing the survey. Knowledge was higher among older women, and those with children and/or tertiary education. More than 90% felt that it was important for young girls to be vaccinated because HPV causes cervical cancer. 73% believed that the benefits of vaccinating girls outweighed any safety concerns. However, one-quarter considered too little was currently known about the vaccination to justify the programme. In addition, 17% were not content with the age at which the vaccine would be administered to girls, 26% had concerns about long-term side-effects and 14% felt that vaccination could encourage unprotected sex. 88% believed that boys should be included in the programme. As regards vaccination of women, two-thirds said they would be likely to obtain the vaccine if it was available for older women. This figure was higher among younger women, but cost was a major disincentive for women of all ages. The findings of this survey reveal gaps in women’s knowledge about HPV vaccination. Although, attitudes were generally positive, the high prevalence of some views could provide a challenge to the national programme. Further analysis will include all respondents and investigate predictors of positive and negative attitudes towards vaccination.

HPV VACCINATION: KNOWLEDGE, PRACTICE AND BEHAVIOURAL INTENTIONS ABOUT PREVENTION OF CERVICAL CANCER AND STD IN FRENCH ADOLESCENTS
Lutringer-Magnin D.1,5 Kaleczinski J.2,6 Barone G1,5 Régnier V.2,6 Leocmach Y.3, Soubeynard B.3, Haesebaert J.1,5 Vanheems P.4,5 Chauvin F.2,6 Lasset C.1,5
1. Centre Léon Bérard, Lyon, France. - 2. Institut de Cancérologie de la Loire, Saint-Étienne, France
5. Université Lyon 1, CNRS UMR 5558, Lyon, France - 6. Université Jean Monnet de Saint-Étienne, IFR 143, Saint-Étienne, France.

Objectives: To examine knowledge about Cervical Cancer (CC) and HPV vaccination and behaviour toward STD among 14-23 years old (yo) girls and determine the correlation with HPV vaccination status.

Methods: From 11/2008 to 04/2009, 316 girls from Rhône-Alpes region were recruited by general practitioners in a cross-sectional study and filled a self-administered questionnaire on CC prevention. Twenty-eight (15 from underserved areas) were interviewed by a sociologist.

Conclusions: Of the 316 girls, 129 (40.8%) were 14-16 yo, 134 (42.4%) were 17-20 yo and 53 (16.8%) were 21-23 yo. HPV vaccination was reported by 135 (42.7%) girls (14-16: 51.2%, 17-20: 44%, 21-23: 18.9%). Parents were involved in the decision of vaccination of their daughters for 69.6% of the 14-16 yo and 54% of the 17-20 yo girls. Among unvaccinated girls, 24.3% intended to get the vaccine soon, 24.3% did not feel concerned, 30.4% preferred to wait, 11.6% were against the vaccine (non response rate: 11%). During the interview, 10 vaccinated girls declared having the same opinion as their parents and uncertain girls thought they were not yet concerned. Even if 90% of unvaccinated girls were aware of HPV vaccine, vaccinated girls were more often informed by family or GP than others (58.5% and 75.6% vs. 28.7% and 50.3% respectively). Knowledge of target of HPV vaccine, PSS purpose, need of PSS despite any safety concerns. However, one-quarter considered too little was currently known about the vaccination to justify the programme. In addition, 17% were not content with the age at which the vaccine would be administered to girls, 26% had concerns about long-term side-effects and 14% felt that vaccination could encourage unprotected sex. 88% believed that boys should be included in the programme. As regards vaccination of women, two-thirds said they would be likely to obtain the vaccine if it was available for older women. This figure was higher among younger women, but cost was a major disincentive for women of all ages. The findings of this survey reveal gaps in women’s knowledge about HPV vaccination. Although, attitudes were generally positive, the high prevalence of some views could provide a challenge to the national programme. Further analysis will include all respondents and investigate predictors of positive and negative attitudes towards vaccination.
DOES UNIVERSITY STUDENTS KNOWLEDGE INFLUENCE HPV VACCINATION? IMPLICATIONS IN HEALTH CARE PROGRAMS

Ramada Diana 1, Medeiros Rui 2

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Objectives: Cervical cancer is an important global public health problem [1]. The major risk factor for the development of cervical cancer is the infection with oncogenic types of human papillomavirus (HPV), one of the most common sexually transmitted infections [2,3]. Portugal has the highest incidence of cervical cancer within the western European Union [4]. One can only take measures for health promotion, disease prevention and screening, regarding cervical cancer, with actual and accurate knowledge about the awareness and beliefs of the population towards this disease [1,5,6]. Knowledge about HPV and cervical cancer depends on several factors such as gender and education, which brings implications for health strategies and vaccination [1]. As educators and caregivers health professionals have an important role in health education programs. To assess the knowledge of future health professionals about HPV and cervical cancer is fundamental to plan accurate and effective health intervention programs that aim the promotion of HPV vaccination and the reduction of cervical cancer prevalence.

Methods: A survey was conducted in Portugal with a representative sample of 1706 university students, from June 2007 to June 2008, in the University of Porto. The students were classified according to their subject of study: Health Sciences Students Vs non-Health Sciences Students. Only health sciences students have formation at curricular level about HPV and cervical cancer. For that, comparisons of knowledge were made analyzing the answers among male and among female students, according to their study area. To verify if there were differences of knowledge between genders it was chosen to make the analysis of the answers in each area separately. Variation of knowledge was also assessed in health sciences students to perceive the type and degree of knowledge that future health professionals have about this matter. The sample was divided in to four categories: male health sciences students, male non-health sciences students, female health sciences students and female non-health sciences students.

Results: In our sample only 55.4% (n=945) students had already heard of HPV. Despite that, only 16.8% (n=287) of the students selected the infection with HPV as being the most frequently sexual transmitted infection, 88.3% (n=834) from that know that it is a risk factor for developing cervical cancer. Although 89% of the students (n=841) wants to be vaccinated against HPV, only 13.8% stated as main reason to be vaccinated “prevention of the disease”. Mean scores of knowledge were calculated. Statistical differences of knowledge were found, regarding “cervical cancer knowledge”, in gender (p<0.001) and between health sciences students and non-health sciences students (p<0.001). Differences regarding the study area in “knowledge and beliefs of HPV” (p<0.001) and in “relation between HPV and cervical cancer” (p<0.001) were also found. Variation of knowledge was found along the courses, in health sciences students, with statistical differences, regarding the specific knowledge about HPV and about cervical cancer: between the beginning/middle (p<0.001) and between the beginning/end (p<0.001) of the courses. Differences regarding the knowledge about the link between HPV and cervical cancer were also found in the beginning/middle (p=0.012) and between the beginning/end (p<0.001) of the courses.

Conclusions: Female students and health sciences students revealed to have more and accurate knowledge than other students. Knowledge of HPV and cervical cancer increases along the courses, in health sciences students: in final year students have moderate knowledge about this matter. These achievements confirm the important role that health professionals and the school/teaching place have in health education. Despite lower levels of knowledge the majority of the students want to be vaccinated against HPV. There are still gaps and misunderstandings of knowledge that may influence sexual behavior and the practice of future health professionals, which may lead to the maintenance of this health problem. To achieve the differences of knowledge in the population may help to develop effective strategies that lead to the decline of cervical cancer incidence and mortality. Educational efforts towards health students are also needed. More effective measures of health education programs need to be applied worldwide to increase awareness and knowledge towards this health problem.

**RELATION BETWEEN VACCINATION STATUS AND TEEN’S KNOWLEDGE ON HPV INFECTION AND VACCINATION**

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**Objectives:** To evaluate if vaccinated teens (VT) are more awareness then no-vaccinated (NVT) about HPV infection and vaccination, and to evaluate the eventual need to develop most effective educational tools.

**Methods:** During June, 2009, we have studied knowledge on HPV in 629 girls, 286 vaccinated (mean age 15.14) – 343 no vaccinated (mean age 16.28) (p<0.001), of secondary school by mean of an anonymous questionnaire, regarding HPV infection and related lesions, HPV transmission and vaccine, sexual/precautionary behaviours after vaccination. For statistical evaluation the t-Student and \( \chi^2 \) tests were performed as appropriated.

**Conclusions:**

98% of VT and 88.6% of NVT have heard about HPV (p<0.001); these 584 girls form the study group (SG). Only 75.4% of VT and 74.7% of NTV report sexual transmission of HPV without add others wrong way. Despite information related to vaccination program, more VT than NVT believe that poor personal hygiene is related to HPV infection (58.6% vs 53.3%, p>0.05). NVT more frequently know that condom use is the only preventive method (VT: 67.5% vs NVT: 78.6% p<0.001), and less frequently consider pills protective against HPV infection (VT: 24.6% vs NVT12.5%, p < 0.001): it may be because NVT were older than VT in the SG.

97.9% of VT and 91.1% of NVT have heard about HPV vaccination (p<0.01). VT know more frequently than NVT that vaccination protects against cervical cancer (98.9% vs 95%, p=0.024), but 22% of VT and 23% of NVT believe that vaccine protects also against other disease not related to HPV; 8.8% of VT think that it protects against HIV infection also. VT more frequently than NVT (96% vs 90%, p=0.023) know that they have to use condom during sexual intercourse out of a stable relationship after vaccination also, but only 75.5% of both groups affirm that they will have pap smear in the future also if they are vaccinated: so 24.5% of VT believe that they will not need pap smear in the future. Our data show that knowledge on HPV infection, vaccination, and sexual and preventive behaviour are not optimal in teens vaccinated against HPV. Without an effective information campaign in the schools, before and after vaccination, it might be an increase in sexual risky behaviour among VT believing that vaccine protects against AIDS, and, in the future, a low compliance to cervix cancer secondary prevention in vaccinated women.

**PREDICTORS OF HPV VACCINE ACCEPTABILITY IN JAPANESE MOTHERS: MAXIMIZING THE PUBLIC HEALTH IMPACT OF HPV VACCINATION IN JAPAN**

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**Objectives:** To determine overall acceptance of HPV vaccines in Japanese mothers and factors associated with vaccine acceptability including knowledge of HPV and cervical cancer.

**Methods:** A total of 2192 mothers with daughters aged 10-14 yrs were recruited from 5 elementary and 14 junior high schools in Sapporo city. After ethical approval, an anonymous questionnaire was distributed in the schools and returned to the main investigator by post between July and Sept. 2010.

**Conclusions:** A total of 876 questionnaires (40%) were returned and 862 were used for analysis. The median age was 42 yrs, 88.1% were married or cohabiting and 60.8% had more than a high school qualification. A total of 61.6% of mothers had been screened for cervical cancer in the past 3 yrs and 12.3% had experiences an abnormal smear. If vaccination were free 92.6% of mothers would vaccinate their daughters, but this decreased to 4.3% if the cost was over 40,000 yen (current price). While 52% of mothers had heard of HPV, only 6.4% knew HPV caused cervical cancer and only 3% thought they had been infected. Similarly, while, 73.1% thought their daughter was at medium-high risk of HPV infection, 72.5% believed their child may die from that infection. While 85.7% wanted more information on the HPV vaccine, 67.6% said they would use the Internet. Only 9.8% would ask a doctor. Factors significantly associated with intent to vaccinate were Pap smear within the past 3 yrs (OR=1.6, 95% CI 1.0-2.7), more than a high school qualification (OR=1.4, 95% CI 1.0-2.3), believing vaccines prevented disease (OR=15.1, 95% CI 6.3-36.5) and not being concerned about childhood vaccine safety (OR=3.8, 95% CI 1.9-7.9). Marital status and history of abnormal smears were not significant. In conclusion, knowledge of HPV is poor. However, high HPV vaccine coverage may be possible if appropriate funding and education are made available.
EW 11

PATTERN OF USE OF HPV VACCINE AND ADHERENCE TO VACCINATION SCHEDULE AMONG INDIVIDUALS EXCLUDED FROM THE PORTUGUESE VACCINATION PROGRAM

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Objectives: To identify and characterize the Human Papillomavirus (HPV) vaccine users profile excluded from the National Vaccination Program, the adverse events (AE) resulting from its administration, the possible sites for vaccine administration and to assess the adherence to vaccination schedule.

Methods: A cross sectional study was undertaken in which pharmacies enrolled invited all users of HPV vaccine from May to July 2009. Invitation letters were sent to all Portuguese pharmacies (n=2669). From June 2009 to February 2010, the subjects who agreed to participate were contacted by telephone for a follow-up evaluation. Data collected included information about the vaccine dispensed, socio-demographic information, age at first intercourse (< or ≥ 18 years-old), data on time at last cervical cytology (of women having ever performed a cervical cytology), sites of vaccine administration, experienced AE and information about adherence to HPV vaccine dosing intervals. Data analysis comprised descriptive statistics and qui-square test or Fisher’s exact test for contingency tables (α=0.05). Cohen’s Kappa was used to measure the agreement between the recommended and the received vaccination schedule.

Conclusions: A total of 309 individuals, recruited from 5.2% (n=138) of pharmacies, participated in the study. Amongst these, 74.8% completed the follow up evaluation. All participants were women, median age of 22 years old and 84.1% had already sexual intercourse. Adherence to intervals and completion of the HPV vaccine 3 doses series were high: 97% received 3 doses of vaccine and the vaccination schedule observed in 73.6% of participants corresponded with the recommended. (kappa = 0.53; p <0.0001). One third of participants reported at least one AE, of which 70.9% were at the injection area. No statistical differences were observed between incident and prevalent HPV vaccine users (p = 0.5061). About half of HPV vaccine doses were administered in health centers and 32% in pharmacies.

EW 12

SAFETY, FEASIBILITY AND ACCEPTABILITY OF VIA AND IMMEDIATE TREATMENT WITH CRYOTHERAPY IN RURAL LAOS

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Objectives: Death resulting from cervical cancer is preventable. In most low-income countries population-based screening is not available or poorly utilized. An alternative test as visual inspection with acetic acid (VIA) followed by immediate treatment was carried out to assess the safety, acceptability and feasibility as a single visit approach for initial cervical cancer screening for rural Lao women.

Methods: From February to August 2009, women of two provinces were recruited for screening using direct visual inspection of the cervix with acetic acid application. If the inspection showed a well defined acetowhite lesion close to the os, immediate cryotherapy was offered.

Results: A total of 1926 women were included, 134 women were tested positive, 113 undertook immediate cryotherapy and none declined treatment. Seventy-seven women returned for one year follow up, and 68 women were VIA negative. There was no report on major complication during and after treatment. Women highly accepted both VIA and cryotherapy.

Conclusion: VIA is a simple, inexpensive, low technology test that requires minimal infrastructure and expenditure. Integrated VIA based screening programs at the primary care level of health services may constitute a feasible method in Laos.
INUIT WOMEN’S ACCEPTANCE AND PREFERENCE OF HPV SELF-SAMPLING IN NUNAVIK, QUEBEC

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Objectives: The circumpolar Inuit population of Canada has a higher incidence of cervical cancer and prevalence of HPV than the general population. A screening program that includes HPV testing on self-collected samples has the potential to increase coverage, but only if self-sampling is found to be acceptable by women in this population. The aim of research was to determine the acceptance and preference towards self-collection of cervicovaginal samples for HPV DNA testing in comparison with provider-collection cervical samples and to explore demographic characteristics of preference for self-collection among a sample of Inuit women from Nunavik.

Methods: Women aged 18-69 years were recruited from a previously formed cohort on the natural history of HPV in Nunavik. Both self-collected and provider-collected specimens were collected with Dacron swabs, and a short written questionnaire was administered to women immediately after specimen collection. Unconditional logistic regression was used to estimate odds ratios and 95% confidence intervals.

Results: Of the 110 eligible women who were approached to participate in this self-sampling sub-study, 85% accepted to participate. Women who accepted had a lower age of first sexual intercourse than women who declined to participate (p<0.001). Self-sampling was preferred by 56% of women. The main reasons for preference towards self-sampling were convenience, privacy, comfort, and ease of test, whereas insecurity in ability to self-sample and lack of comfort with test were the main reasons for preference towards provider-sampling. Educational attainment was the only predictor of sampling method preference, where having at least a grade 9 education was negatively associated with preference towards self-sampling (OR: 0.20, 95% CI: 0.05-0.75). The most common reason for more educated women to prefer provider-sampling was that they were worried about their ability to sample correctly.

Conclusion: Although preference for self-sampling in this population was lower than expected, given the high acceptance rate it may increase coverage, especially if its introduction occurs with educational activities.

ACCEPTABILITY OF HPV SELF-SAMPLING FOR CERVICAL CANCER SCREENING IN A LOW RESOURCE COUNTRY

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Objectives: Cervical cancer is the leading cause of cancer-death among Cameroonian women, mainly because of lack of screening. The purpose of this study was to assess acceptability and preference for self or clinician-sampling.

Methods: Two hundred forty-three women aged between 25 and 65 years old were recruited through a cervical cancer screening campaign conducted from July 7th to 13th at CHU Yaoundé, Cameroon. Eighteen cases were excluded because they did not fit protocol criteria. A written survey was proposed to 217 women after they underwent unsupervised HPV self-sampling followed by a pelvic exam for Pap test performed by a clinician. Pap test was collected using liquid based cytology, allowing cell analysis and HPV testing. Acceptability of HPV self- and clinician-sampling were evaluated as well as knowledge and understanding about cervical cancer.

Conclusions: Median age was 38 years old. A majority of women preferred clinician-sampling (62% vs. 28%; p<0.05), essentially because they felt “more confident” with it. Preference for self-sampling was positively associated with knowledge about cervical cancer (47% vs. 20%) and higher education (34% university graduated vs. 28% for secondary school and 18% for primary school women; Clinician-sampling induced more discomfort, pain and embarrassment than self-sampling (p<0.005 for all the outcomes). In conclusion, women found clinician-sampling for HPV more acceptable than self-sampling. If self-sampling is offered in the future, efforts are needed to address women’s concerns regarding the accuracy of the test. It would also be important to increase knowledge about HPV and cervical cancer.
**EW 15**

**LONG-TERM WORRIES IN WOMEN WITH LOW-GRADE ABNORMAL CYTOLOGY MANAGED BY ALTERNATIVE POLICIES AT COLPOSCOPY: THE TOMBOLA TRIAL**

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**Introduction:** The adverse psychosocial effects on women of receipt of an abnormal cervical cytology result are well documented. Worries about cancer are common. Less is known about the psychosocial impact of different management policies, especially in the longer-term. We investigated cancer and other worries over 3-years in women with low-grade abnormal cytology referred for colposcopy and managed by alternative policies.

**Methods:** 1,515 women, aged 20-59 years, with recent low-grade abnormal cytology, who attended colposcopy, were randomised to (a) immediate large loop excision (LLETZ) or (b) 2-4 punch biopsies with recall for LLETZ if these showed CIN2/3. Women completed questionnaires at recruitment and after 12, 18, 24 and 30 months. These included questions on the extent of any worries about their general health, cervical cancer, having sex and fertility since getting their cytology result. Prevalence of worries was computed for each time-point (point prevalence) and at any time-point during 12-30 months (cumulative prevalence). Multivariate odds ratios (ORs) for immediate LLETZ versus biopsy and selective recall were computed using logistic regression. At recruitment 69% were worried about their general health, 68% about having cancer, 28% about having sex, and 24% about future fertility (63% of those planning to have a child in the future). Although worries declined over time, by 30 months, they were still common (general health, 35%; cancer, 16%; sex, 12%; future fertility, 12% overall and 39% of those planning children). Prevalence of cancer worries was slightly lower in the immediate LLETZ arm, but this was not statistically significant either overall (cumulative: immediate LLETZ, 40%; biopsy & recall, 41%; OR=0.86, 95%CI 0.66-1.10) or at any time-point during 12-30 months (cumulative prevalence). There were no significant differences between arms in the other worries.

**Conclusions:** Substantial proportions of women with low-grade cytology are affected by long-term worries, irrespective of management. Long-term psychological impact should be considered when assessing the costs and benefits of cervical screening.

**EW 16**

**FROM GOOD INTENTIONS TO ACTUAL UPTAKE: A COMPARISON OF PREDICTORS OF HPV VACCINATION IN COLLEGE WOMEN**

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**Objectives:** The objective of this study was to identify predictors of both Human Papillomavirus (HPV) vaccination intentions and uptake with the aid of the Health Belief Model and Theory of Planned Behavior as conceptual frameworks. This study is one of the first to compare the predictors of intentions and uptake.

**Methods:** Undergraduate females (n = 447) who had either received, intended to receive, or did not intend to receive the HPV vaccine were surveyed about factors that influenced either their intentions or actual decision to receive the HPV vaccine. A multivariable logistic regression analysis was used to assess these predictors.

**Conclusions:** Attitudes, subjective norms (e.g., peer or family influences), doctor recommendation and the perception that the vaccine had negative health consequences significantly predicted both vaccine intentions and actual uptake. Additionally, perceived behavioral control (regarding health issues) and perceived susceptibility to infection predicted uptake only. The strongest predictors for uptake were doctor’s recommendation and subjective norms. The approval of peers and parents concerning HPV vaccine decision-making, as well as physician’s recommendations may have a particularly strong impact in transforming intentions into actual vaccine uptake. This study further suggests that vaccine decision-making is a complex process influenced by multiple factors. Future interventions and media campaigns should target young women’s perceived barriers, attitudes and personal sense of control, coupled with credible medical and/or peer endorsements to enhance vaccine uptake.
In the United States, the age-adjusted incidence rate for cervical cancer was 8.1 per 100,000 women per year, and the age-adjusted death rate was 2.4 per 100,000 women per year. Infection with a high risk type of the human papilloma virus (HPV) is a necessary though not sufficient cause of the vast majority of cervical cancers. With the approval by the U.S. Food and Drug Administration of both the quadrivalent and bivalent HPV vaccines, published recommendations by the Advisory Committee on Immunization Practices for use of the vaccine among women (9-26 years) and permission for vaccination of boys and young men, dissemination of the vaccine is critical in reducing HPV disease-risk burden. This state of the science review focuses on the uptake of the bi- and quadrivalent HPV vaccines among girls and young women in the US, with comparisons to developed and developing countries. Why focus on young women and girls alone? More population-level data are available and they are at risk for one of the most serious HPV-related diseases, cervical cancer. The epidemiology of HPV, the mechanisms of action, protocols for vaccine inoculation, rates of uptake, and barriers to vaccination at the policy, provider, and patient levels are reviewed. Policy-, provider-, parent-, and patient-directed interventions are described. Legislation supporting mandates, and subsidized public education are explored at the policy level. Clinical counseling approaches to patients and their parents, concurrent immunizations to adolescents, prevention visits, and the dissemination of academic detailing are explored to increase uptake particularly in under-resourced communities and countries. Strategies to increase patient education among parents, girls and young women are examined. Population-based surveillance is approaches to developing robust HPV estimates are described. Recommendations for additional research to comprehensively examine socio-demographic, psychosocial, and socio-economic factors that predict actual vaccine uptake according to the protocol among age-eligible girls and young women--as well as young men and boys--are proposed.

Background: In Japan, the bivalent HPV vaccine was approved in October, 2009, and became available as a non-routine vaccine from December, 2009. National vaccination programs are done based on the Preventive Vaccination Law and are not usually school-based. Routine vaccinations are free and funded by national and regional governments. However, the cost and responsibility for non-routine vaccinations are left to individuals. In exceptional circumstances regional government fund non-routine vaccinations. This was the case in Shiki City, Saitama Prefecture, where a high uptake rate was obtained.

Materials: On January 20, 2010, the mayor of Shiki City announced to the media his decision to vaccinate adolescent girls in Shiki City against HPV. A project team for HPV vaccination was set up in the city’s health promotion center. To gain mutual consent for HPV vaccination, senior health professionals, city officials, the head of the board of education, school principals and health-care teachers in elementary and junior high schools met several times. The cohort to be vaccinated was 1,254 girls aged 12 to 15 years. Individual notifications (invitations) were mailed to each girl and their parents on April 23, 2010, along with information about the HPV vaccine, the vaccine coupon and a list of medical institution where the vaccine was being offered.

Conclusions: As of December 28th, 2010, the uptake rate for girls aged 15 year old was 88% (88.3% and 87.3% for the 1st and 2nd dose, respectively). The vaccine registry is managed by the health care system of the city. The activities of Shiki City were reported in newspapers and on the television, etc. and many people learnt about the HPV vaccine. The success of the HPV vaccination program and high uptake rates in Shiki city may be a good model for the nationwide HPV vaccination program that will start in 2011.
FREQUENCY OF HPV GENOTYPES IN WOMEN WITH NORMAL CERVICAL CYTOLOGY IN PARANÁ STATE, BRAZIL

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Objectives: Human papillomavirus (HPV) infection is a causative factor for cervical cancer. Early detection of high risk HPV types might help to identify women at high risk of cervical cancer. The aim of the present study was to examine the HPV frequency and genotypes in women with normal cervical cytology in Paraná State, Brazil.

Methods: A total of 418 women were studied. All women had normal cervical citology. Through PCR and RFLP techniques it was possible to study 40 HPV types: high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 e 82), undetermined-risk (26, 53 e 66) and low risk (6, 11, 30, 34, 40, 42, 43, 44, 54, 55, 61, 62, 64, 67, 69, 70, 72, 74, 81, 83, 84 e 91). Also sociodemographic characteristics and sexual behavior were recorded.

Conclusions: HPV was detected in 6.7% of the study population, mainly in women aged < 25 years. The overall prevalence of high-risk, low-risk, undetermined-risk HPV types and co-infections was 36%, 29%, 10.7% and 25% respectively. HPV-16 was the most common type detected (17.9%) followed by HPV-31 (10.7%) and HPV-70 (10.7%). Detection of HPV DNA was significantly associated in the bivariate analysis with number of sexual partners within the last 12 months (p<0.031; OR= 5.4; CI=0.98-29.8). The HPV frequency in women with normal cervical cytology in Paraná State was relatively low. In contrast, the number of infected women with HR HPV was high, especially HPV 16. Our results demonstrate the importance of studies including this type of sample, mainly in Brazil, where such studies are scarce.

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GENOTYPES OF HUMAN PAPILLOMA VIRUS IN SUDANESE WOMEN WITH CERVICAL PATHOLOGY


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Objectives: Knowledge of the distribution of human papillomavirus (HPV) genotypes among women with cervical lesion and in invasive cervical cancer is crucial to guide the introduction of prophylactic vaccines. There is no published data concerning HPV and cervical abnormalities in Sudan. Aim: This study aimed to define the prevalence of HPV and its subtypes in the cervical smears of women presenting for routine gynecology examination at Omdurman Military Hospital, Sudan.

Methods: During the period between March 2003 and April 2004, 135 cervical smears collected from these women were screened using cytological techniques, and analysed by PCR for (beta)-globin and HPV DNA using gel electrophoresis and ELISA. Of these 135 smears, there were 94 (69.3%) negative, 22 (16.3 %) positive for inflammation, 12(8.9) mild dyskaryosis, 5 (3.7) moderate dyskaryosis and 2 (1.8) severe dyskaryosis. There were 60.7% β. globin positive samples for HPV indicating DNA integrity. HPV DNA was identified in three samples (2.2%) by gel electrophoresis and. was positive in four samples (2.9%) as single and multiple infections by PCR-ELISA. The high risk HPV types 16 and 58 were identified in one sample as a mixed infection. The low risk HPV types 40 and 42 were also found as a mixed infection in another patient. HPV types 58 and 42 were identified in the other two patients.

Conclusions: HPV type distribution in Sudan appears to differ from that in other countries. The HPV genotypes identified were not associated with cancer.
INCIDENCE RATES OF GENITAL WARTS IN YOUNG WOMEN AND MEN IN FRANCE

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Objectives: The number of diagnoses of genital warts (GW) has gradually increased over the last ten years in most Western countries. Vaccines for genital human papillomavirus (HPV) have been licensed for use. Our objective was to assess the impact of the HPV vaccination programme on the incidence of GW in women and men and to describe the sexual behavior of these patients. Baseline results of the study are presented.

Methods: A prospective study was conducted through a representative sample of gynaecologists and dermatologists. Investigators enrolled during a 4-month period women aged 15-26 and men aged 20-30 presenting a first occurrence of GW. A parallel evaluation of the incidence of genital herpes was performed. Data on demographics, GW diagnosis and sexual behaviors were collected. Incidence rates of first occurrence GW were estimated by extrapolation for a 12-month period and the national number of gynaecologists or dermatologists.

Conclusions: 161 gynaecologists and 314 dermatologists participated; 181 first occurrences of GW were reported among women and 589 among men. The number of patients included per week by each physician was constant throughout the 4-month analysis period. Mean age of women was 22.2 ± 2.8 years and of men was 25.5 ± 3.0 years. First sexual relations occurred at a mean age of 17.2 ± 2.2 for women. The majority of women had had up to four sexual partners since being sexually active while the majority of men reported having had at least five sexual partners. Condom use was scarce (91% of women and 83% of men used condoms sometimes or never). The estimated annual incidences of GW in women aged 15-26 and men aged 20-30 were 343/100,000 [95% CI: 327.5-494.5] (corresponding to 23,027 new cases) and 528/100,000 [95% CI: 487-568] (corresponding to 23,027 new cases), respectively. Genital herpes incidences in women aged 15-26 and men 20-30 were 384/100,000 [95% CI: 327-440] and 87/100,000 [95% CI: 72 – 104], respectively.

We report results of the first study in France analysing results on the incidence of GW in women and men. These findings suggest that GW have a substantial burden in France in women and men. These data will allow assessing the impact of HPV vaccination in the next years.

HUMAN PAPILLOMAVIRUS GENOTYPE DISTRIBUTION IN TONSIL CANCERS

Lacau St Guily, J1; Clavel, C2; Okaïs, C3; Prétet, JL4; Beby-Defaux, A3; Agius, G4; Birembaut, P5; Jacquard, AC5; Léocmach, Y3; Soubeyrand, B3; Riethmuller, D4; Denis, F6; Mougin, C6
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5. Laboratoire de Virologie, CHU Poitiers, Univ Poitiers, France - 6. CHU Dupuytren, Limoges, France

Objective: Among Head and Neck cancers, the incidence of tonsil cancers has been gradually increasing over the last decades and recent studies showed an association between tonsil cancers and human papillomavirus (HPV) infection. French data on HPV prevalence in tonsil cancers are scarce. The objective of this study was to assess the overall and type specific HPV prevalence in tonsil cancer histological samples.

Methods: Tonsil invasive cancers diagnosed between 2000 and 2009 in 12 French centres were retrospectively collected and centrally tested for HPV genotyping (INNO-LiPA assay allowing the detection of 28 genotypes).

Results and Conclusion: A total of 220 of histological samples of tonsil invasive cancers were collected. 84% (185/220) were included in the study while 16% were excluded from the analysis because neither HPV nor cellular DNA could have been amplified. Mean age at diagnosis was 60 ± 10.5 years. There were 81% (150/185) of males. We found an overall HPV prevalence of 57% (106/185). Mean age at diagnosis was comparable in HPV positive tonsil cancer cases (60 ± 11.2) and HPV negative tonsil cancer cases (59 ± 9.6). HPV prevalence was higher in females than in males (80% versus 52% respectively). Among HPV positive tonsil cancer cases 92% (98/106) were infected by a single HPV type and 8% (8/106) were infected by more than one HPV type. HPV 16, and HPV 16 and/or 18, were detected in 89% (94/106) and in 91% (96/106) of HPV positive tonsil cases, respectively. All other HPV types had prevalence below 5%.

Our results confirm that HPV is common in tonsil cancers and also emphasize the predominant role of HPV 16 in France. Unlike cervical cancer, the association of HPV and oncogenic mechanisms remains to be clarified. These results are an indicator of the HPV distribution in tonsil cancers before HPV vaccine implementation among women. The current HPV vaccination of young women will be able to contribute a posteriori to clarify the possible causal association between tonsil cancers and HPV.
CERVICAL NEOPLASIA IN PREGNANCY - CASE REPORT AND REVIEW

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Objective: To present a case report about cervical cancer during pregnancy and review of all the cases diagnosed in our institution.

Methods: Retrospective study based on clinical files consultation. We describe in detail the latest case diagnosed and review the other cases of cervical cancer diagnosed during pregnancy, according to diagnostic pathway, weeks of pregnancy and stage at the diagnosis, treatment and newborn outcome.

Case: In a routine vaginal examination, in a thirty-four years old caucasian women, 16 weeks pregnant, we identified a cervix suspicious lesion in which, under colposcopic control, multiple biopsies in major lesions areas were preformed. She had no previous pap smear results. Histological examination diagnosed a squamous cell cervical cancer. Thoracic, abdominal and pelvis imaging evaluation was realized, and neoplasia was staged as IB1. Woman decided to proceed with pregnancy and, at 34 weeks, after pulmonary maturation, an elective caesarean section and a radical hysterectomy were performed. She delivered a girl, 2935g, Apgar Index 9/10. Histological evaluation of surgical specimen confirmed stage IB1. This patient is now under follow-up. No adjuvant therapy was needed.

Review: In our institution two other cervical neoplasias were identified. Like this case, the other two were diagnosed in the second trimester (16 weeks) in a black woman with 29 years and a 33 years old caucasian woman. They differ from this case because the diagnostic pathway included first an abnormal pap smear (ASC-US and HSIL) and only after a colposcopic evaluation with biopsy and histological evaluation. They were both stage IB1. One woman decided to interrupt the pregnancy and at the same time a radical hysterectomy was performed. The other woman decided to proceed with the pregnancy and, like the case described, an elective caesarean section and radical hysterectomy were performed at 34 weeks, with a male newborn, 2346g, Apgar Index 8/9. Both women are alive without disease.

Conclusion: Pregnancy offers a unique opportunity for the early diagnosis of cervical cancer, due to the fact that pregnant patients have many possibilities of gynaecological and cytological examinations. Treatment decision depends on cancer stage and women will.

DISTRIBUTION OF HUMAN PAPILLOMAVIRUS GENOTYPES IN WOMEN WITH ABNORMAL PAP TESTS CLASSIFIED ACCORDING TO BETHESDA 2001

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Objective: The purpose of this study was to determine distribution of human papillomavirus (HPV) genotypes in the spectrum of cytological abnormalities of squamous cervical epithelium detected by Pap test.

Methods: Cervical samples of 333 patients with cytological abnormalities of squamous cells were analysed: ASCUS (N=58), LSIL (N=72), ASC-H (N=30), HSIL/CIN2 (N=67), HSIL/CIN3 (N=80) and squamous cell carcinoma (SCC) (N=26). All samples were previously positive to high risk probe of hybrid capture 2 HPV DNA test (Qiagen) and analysed by short chain polymerase chain reaction reverse hybridization line probe assay (Inno-LiPA) detecting 26 different HPV genotypes.

Results: Infection with single HPV genotype was found in 76% of ASCUS, 60% of LSIL, 70% of ASC-H, 60% of HSIL/CIN2, 73% of HSIL/CIN3 and in 73% of SCC. Infection with two or more HPV genotypes was found in 21% of ASCUS, 39% of LSIL, 23% of ASC-H, 39% of HSIL/CIN2, 26% of HSIL/CIN2 and in 20% of SCC. HPV was not detected in 3% of ASCUS, 1% of LSIL, 7% of ASC-H, 1% of HSIL/CIN2, 1% of HSIL/CIN3 and 7% of SCC (two cases). The most frequent HPV types detected in the ASCUS group were: 16 (19%), 31 (19%), 52 (14%), 45 (9%) and 66 (9%); in LSIL: 51 (20%), 52 (16%), 16 (15%), 56 (11%) and 53 (11%); in ASC-H: 16 (30%), 31 (20%), 18 (10%), 45 (10%) and 52 (10%), in HSIL/CIN2: 16 (28%), 31 (16%), 33 (13%), 52 (12%) and 18 (10%); in HSIL/CIN3: 16 (42%), 31 (19%), 33 (13%), 35 (6%) and 52 (6%); in SCC: 16 (58%), 31 (11%), 45 (11%) and 52 (8%). The most frequent HPV type detected in all groups was HPV 16 (30%) followed by 31, 52 and 33. In the low grade lesions (ASCUS+LSIL) HPV 16 was found in 17% of cases, and in the high grade lesions (ASC-H+ HSIL+ SCC) was found in 38% of cases. HPV 18 was present in 5.7% of all cases, 3% in low grade and 7.4% in high grade lesions.

Conclusion: The most frequent detected HPV genotype was HPV 16 followed by HPV 31 in all groups but LSIL which showed predominance of HPV 51 and 52. HPV genotype 18 was underrepresented in all groups. These results could represent valuable data for the future planning and follow up strategies in the cervical cancer prevention programmes.
**EVALUATION OF PREDICTIVE VALUE OF NEW SEX PARTNERS AS A PREDICTOR OF PREVALENT HPV INFECTION, HR-HPV AND PREVALENCE OF HPV TYPE 16 IN WOMEN AGED 15-25 YEARS**

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**Objective:** A first peak in HPV prevalence in young women aged 18-20 has been reported. A number of factors has been already estimated in order to examine the predictive value of each one. The aim of this study is to examine a new parameter - the new sexual partner and the number of new sexual partners recorded in the last six months in relation of prevalence of HR-HPV and of prevalence of HPV 16 among women 15-25 yrs old.

**Methods:** We are enrolling women age 15-25 years attending St. Savvas GYN clinics for routine screening during 2008-2010. Detailed information on demographics, sexual, menstrual, and reproductive history, and medication use is recorded. Cervical swabs were tested for HPV using Papillocheck HPV genotyping assay (Greiner). In the interim analysis of 254 women with normal cytological diagnosis aged 15-25 yrs, the overall prevalence of HPV infection was 23.2%. The prevalence of HR-HPV was 5.9% and the prevalence of HPV type 16 was 2.66%. The risk of HPV infection among women reporting a new male sex partner was higher (adj OR 24.7, 95% CI 4.4-138) versus women with no new partner in the last 6 months. HR-HPV prevalence increased with increased number of new sexual partners during the last 6 months, the odds of HR-HPV were higher in women who reported > 2 new sexual partners in the last 6 months (adj OR 5.0, 95% CI 1.5-17.0) compared to women with no new sexual partners. The dominant type of HPV in this group was HPV 16.

**Conclusions:** While young women reporting new sexual partners in the last 6 months have a high risk of HPV infection, and higher risk of HR-HPV. The high number of sexual partners emerged as a strong risk factor for HR-HPV infection independent of sexual behavior.

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**GENOTYPE SPECIFIC CONCORDANCE OF GENITAL HPV INFECTIONS IN MALES AND THEIR SPOUSES IN FINNISH FAMILY HPV STUDY**

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**Objectives:** Genotype-specific concordance among HPV-infected spouses is incompletely assessed. HPV concordance is of importance in understanding viral transmission modes and is necessary for providing adequate counselling for HPV-infected partners.

The aim of this study was to establish the genotype specific prevalence and distribution of genital HPV infections in males and to determine the HPV concordance among spouses participating in the Finnish Family HPV study.

**Methods:** Urethral and semen samples were taken from 131 fathers-to-be and their pregnant spouses at their 3rd trimester. HPV genotyping was done with Multimetrix kit (Progen, Heidelberg, Germany). The male HPV data at baseline were correlated with the HPV results from cervical and oral mucosal samples of the spouses at baseline.

**Results:** Urethral and/or semen samples tested HPV positive in 41/87 (46%) of men. HPV DNA was detected more in semen than in urethral samples (31% versus 23%). In women 19% of the genital and 17% of the oral samples tested HPV positive. Of the HPV genotypes, HPV16 was the most frequent one, present in 39% of semen-, 77% of female oral- and 29% of female genital samples. Multiple-type infections were found in 24% of HPV-positive urethral and 22% of semen samples. In the spouses, half of the genital HPV infections and 4.5% of oral infections were multiple. The HPV genotype-specific concordance among spouses ranged from 0% to 9.5%, according to the sampling sites. Altogether, 8 couples disclosing such a concordance were analysed separately for a risk-profile. Mothers of the HPV-concordant couples reported significantly higher number (>6) of life-time sexual partners than did the mothers in discordant couples (p=0.030).

**Conclusions:** In the Finnish Family HPV study, asymptomatic HPV infections were common in both spouses and the genotype-specific concordance among the spouses was low. HPV16 plays a dominant role. Of note is that HPV6 is a common type in men, having implications e.g. in design of prophylactic HPV vaccination programmes.
PREVALENCE OF GENITAL HPV IN WOMEN AND CORRELATION WITH OTHER GENITAL INFECTIONS IN LISBON AND VALE OF TEJO

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Objective: Analyze the prevalence of the genital infection by Human Papiloma Virus (HPV), characterize genotypically and determine correlation with infection by Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma spp.

Methods: Transverse study made with 462 sample`s of women (16-65 years) from the Gynecology consultation of British Hospital XXI, between December of 2009 and October 2010. Cervical specimens were obtained, in medical consultation, for cytology, HPV, Chlamydia trachomatis and Mycoplasma hominis and Ureaplasma spp. detection.

For determination of HPV the method used was Polimerase Chain Reaction and for detection of mRNA of the oncogene E6/E7 the method used was TMA® (Gen-Probe®). The technique used for the research of Chlamydia trachomatis was Enzyme Liked Fluorescent Assay (ELFA), and the research of Mycoplasma hominis and Ureaplasma spp. was made in a cultural medium.

Results: Our study presents a total of 89 positive cases with a HPV high risk prevalence of 84%, and age distribution was as follows: 5% (< 20 years), 33% (20-30 years), 31% (30-40 years), 8% (40-50 years) and of 7% (50-65 years). Subjects with co-infections for HPV reached a percentage of 30%. High-risk genital HPV infection was 13% in normal cytology. The oncoprotein determination E6/E7 was made in 23 subjects and 74% of these were positive. Infections by Chlamydia trachomatis were not detected and 1% of the studied population presented infection for Mycoplasma hominis and 11% for Ureaplasma spp.

Conclusions: In our study the average age of infection is 35 years. High risk HPV prevalence was high, and the majority of the subjects had oncoprotein expression E6/E7 positive increasing the predisposition for the development of cervical cancer. There was no correlation between HPV infection and Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma spp. infections.

ASC-US: IS AGE A RISK FACTOR FOR THE DEVELOPMENT OF CIN?

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Introduction: Cervical cancer is the most common gynecologic malignancy in the world. This type of cancer has a known natural history of lesions that are considered is precursors. These precancerous lesions are epithelial cells abnormalities that can be identified by cytology. One of the cytologic finding is called atypical squamous cells of undetermined significance. In half the cases no histological abnormality is found. In these women a negative HPV test excludes other exams as colposcopy. Nonetheless 10 to 20% of women with ASC-US have a biopsy result of CIN 2 or higher.

Objective: Evaluate if age is a risk factor for this citologic abnormality and how it influences the progression for cervical intraepithelial neoplasia.

Methods: Retrospective study of the histopathological and colposcopic findings in women referred for colposcopic examination in our hospital between January 2006 and December 2010 with the result of ASC-US in cytology. We divided our patients in groups according to age: <20, 21-30, 31-40, 41-50, 51-60, 61-70, >70.

Results: In the period studied, 6730 colposcopies were done in our unit. 5% of these corresponded to women referred, due to a ASC-US result in there cytology. The majority of women were in the group of age of 31-40 and 41-50. In most cases, colpocopy showed a normal cervix. Further results will be presented latter.

Conclusion: The prevention of cervical cancer is the key factor for the reduction of the mortality and morbidity of this disease. A correct follow-up of the women with cytological abnormalities such as ASC-US is essential as a better knowledge of the risk factors that influence the progression of ASC-US to neoplasia. Age is one of these factors.
The cervix cancer is a cancer that can be prevented. The prevalence in Portugal is 14/100,000 cases. Regular screening of woman (Papanicolaou + HPV) is the most efficient way to prevent this cancer.

**Objective:** Determined the prevalence of HPV and others sexually transmitted agents in a population from 6 Health Units of Santa Casa Misericórdia, Lisbon District.

**Methods:** From a population of 8875 women, 3841 were analysed cytologically. From these, 453 samples (average age: 40.2y) were analysed randomly for HPV. The samples were collected for PreservCyt Solution and processed in the ThinPrep processor. All slides were stained with Papanicolaou and evaluated according to the Bethesda 2001 ed. HPV presence was evaluated by In House qPCR (SPF primers). HPV positive samples were typed using Papillocheck and INNO-LIPA.

**Results:** In cytological results, 87.7% of these women were negative for neoplasia and 12.3% had cytological alterations: ASC-US (3.4%); ASC-H (1.1%); LSIL (4.0%); HSIL (2.2%) and AGC SOE (1.6%). 27.7% had the presence of microorganisms (15.5%): *Thrycomonas vaginalis* (TV-1.8%), *actinomyces* (0.2%), fungi (3.6%) and flora deviation (9.9%). Reactive reactions were reported in 11.7% of the cases: atrophy (2.5%) and inflammation (9.2%). The HPV detection was performed in 446 samples: 47.8% were positive for HPV. The prevalence of HPV by cytological result were 45% of positivity in negative woman, 53% in ASC-US, 60% in ASC-H, 89% in LSIL, 90% in HSIL and 29% in AGC SOE. The more prevalent types were HPV 16 (22.5%), HPV 56 (4.7%) and HPV 31 (3.8%).

**Conclusions:** The prevalence of HPV (47.8%) were very high when compared with the literature. This result can be justified by the higher incidence of abnormal cytology: 4.0% with LSIL and 2.2% with HSIL, where the expected values range between 1.4-2.8% (LSIL) and 0.3-0.8% (HSIL). By other hand, the TV cases are too high when compared with the expected values: 1.8% vs 0.37-0.54%.

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**THE INCIDENCE OF HUMAN PAPILLOMA VIRUS (HPV) INFECTION IN THE POLISH ARMY POPULATION AND ITS CORRELATION WITH SELECTED PARAMETERS OF IMMUNOLOGICAL SYSTEM**

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**Background:** In Poland since 1988 women have been allowed to serve in professional army. The number of women taking this opportunity is continually growing. Admitting women to military service triggers the need to conduct research into problems which occur together with their presence in the army. Taking into account young age of both soldier females and males, staying in closed environment dominated by men carries the risk of increased incidence of sexually transmitted diseases, including HPV infection.

**Objective:** Evaluation of HPV infection incidence in men and women serving in the Polish army together with immunological system parameters investigation.

Study Design: In years 2009-10 the research was performed into HPV incidence in a group of 243 potentially healthy soldiers (148 women and 95 men) aged between 25 and 30. In order to identify HPV infections, DNA extracted from cervical squamous epithelial cells was tested by PCR. Moreover, immunological condition of participants was checked by measuring blood serum: IL-2,4,18, IFN gamma, TNF alfa, and IgG,A,M,E antibodies concentration.

**Results:** In this group of 243 soldiers high risk HPV infection was confirmed in the first examination in 10% of women and men, low risk HPV was confirmed in 1.1%, and 88.9% of soldiers were negative. High risk HPV infection was twice as common in women compared to men, but lower risk HPV was almost two and a half times common in men than in women. Additionally, in the test repeated after half a year the numbers were: 7.8% (high risk), 5.6% (low risk) and 86.7% (negative). There were no connection between HPV type and immunologic parameters.

**Conclusion:** Despite young age and staying in closed environment the incidence of HPV infection in the army corresponds to its frequency in overall population in Poland. Also, in the conducted research, no correlation was observed between the type of HPV infection and changes in investigated immunological parameters.
DIFFERENCE OF HPV GENOTYPING IN CERVICAL PRECANCER AND CANCER

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Objective: The objective of the study was to compare the distribution of HPV genotypes in glandular and squamous cervical precancer and cancer.

Methods: 2213 women diagnosed with cervical intraepithelial neoplasia 2-3 (CIN 2-3), 614 women with squamous cell carcinoma (SCC), 60 women with adenocarcinoma in situ (AIS), and 97 women with adenocarcinoma (ADC) were enrolled in this study. Samples obtained from liquid-based cytology were analyzed for HPV genotyping using HPV DNA chip test and other samples were underwent Hybrid capture II system. The HPV DNA chip test harbor 24 HPV probes (15 high-risk types and 9 low-risk types) and has the advantage of being able to detect 24 HPV types simultaneously.

RESULTS: The most common HPV types in CIN2-3 were HPV16, 58, 33, 31 and 18(37.1%, 15.8%, 8.2%, 7.7%, 6.6%, respectively). In SCC the most common types were HPV 16, 18, 58, 33, and 31(54.1%, 10.6%, 9.6%, 5.4%, 5.0%). In AIS, HPV 16,18,45,53 were 40%, 40%, 3.3%, 3.3% respectively. In ADC, most common genotypes were HPV 18(54.6%), 16(27.8%) and 45(7.2%). The proportion of samples that resulted in negative of HPV by Hybrid capture II system in ADC was 20.9% compared to 11.8% in SCC(P<0.01).

Conclusion: There was a difference between common HPV genotypes in squamous cell carcinoma and adenocarcinoma. In Adenocarcinoma, negativity of HPV was common than squamous cell carcinoma which suggests that adenocarcinoma may have a different carcinogenesis from squamous cell cancer other than HPV infection.

ABSTRACTS

HUMAN PAPILOMAVIRUS DISEASE BURDEN IN PORTUGAL

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Objectives: Human Papillomavirus (HPV) is responsible for the most common sexually transmitted infections. Around 70% of individuals will contact with HPV through life. Although most HPV infections usually clear spontaneously within 2 years, it is associated to a wide spectrum of diseases that include cervical, vulvar, vaginal, anal, pre-cancerous and cancers and genital warts. HPV types 16 and 18 are responsible for around 70% of cervical cancer cases, 80% of adenocarcinoma in situ, 45-70% of cervical pre-cancer-lesions, 70% of vulvar and vaginal pre-cancer lesions. HPV types 6/11 are responsible for 90% of genital warts. HPV types 16/18 are also responsible for other cancers (penile, head & neck) but to a lesser extent. Most recent National Oncological Registry (2005) attributes to HPV the following number of new cases in Portugal: 614 cervical, 81 vulvar, 28 vaginal, 83 anal and 76 penile cancers, a reality clearly under evaluated.

Data on genital warts point to around 9.000 new cases/year only in female gender.

The objective of the present study is to capture the real burden of HPV related diseases in Portugal, focusing on HPV types 6, 11, 16 and 18 included in the marketed vaccines, in terms of cases and costs to the healthcare system and the society

Methods: To estimate the incidence of cancer cases we used an iterative methodology with hospital data from DRGs (Diagnosis Related Groups) and the application of Disease Staging System. Due to the lack of official data, a mixed strategy with international incidence data and Portuguese data from a set of health institutions was used, to estimate the incidence of genital warts.

Mortality data considered used Directorate-General of Health data (DGS).

Regarding financial burden, unit and average costs per procedure were obtained from national DRGs official fees. The treatment costs estimation of cancer included 3 to 5 years (depending of the type) and for pre-cancerous lesions used 3 years, considering hospital and disease staging information. For genital warts costs were estimated through an expert panel.

Conclusions: HPV related diseases represent an enormous burden in terms of morbidity and mortality in Portugal. Diagnosis, management and treatment of HPV related diseases cause significant costs for the healthcare system and society. Further analyses are still ongoing to quantify detailed costs by associated HPV genital disease, gender and level of care.
ASC-H: TREATMENT AND RECURRENCE POST-LOOP ELECTROSURGICAL EXCISION PROCEDURE (LEEP)

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Objectives: To evaluate the histology of the LEEP surgical tissues of patients with ASC-H and post-LEEP recurrence.

Methods: Medical records of patients with ASC-H treated with LEEP between January 2004 and March 2008 in the town of União da Vitória, Paraná, seat of the Sixth Public Health Region of Paraná (CISVALI), were evaluated. The LEEP was carried out solely for ASC-H immediately after colposcopy, but without a histological diagnosis.

Conclusions: Most patients were less than 40 years old (71.1%), with the largest group 20-39 years old (p< 0.0001). Twenty-eight patients (73.3%) showed histological lesions. CIN I was present in 7 (18.4%), CIN II and CIN III in 9 (23.7%) each, microinvasive squamous cell carcinoma (SCMCA) in 2 (5.3%), and SCMCA plus in situ adenocarcinoma (ISAD) in 1 (2.2%). In 32 patients (84.2%), there was no involvement of the margins, including 100% with no dysplasia histology and CIN I, 80.0% of those with CIN II, and 88.9% of those with CIN III. Two patients (5.3%) had endocervical involvement, all of them with CIN II. Four patients (10.5%) had ectocervical and endocervical involvement, 1 of them with CIN III, and 3 of them with carcinomas. All patients with follow-up (+) were ASC-US, with no patients with dysplasia or CIN I. Conclusions A very high portion of the women with ASC-H had lesions on post-LEEP histological examination, principally CIN II and III. These data show the benefits of treatment for ASC-H by LEEP immediately after colposcopy but without any previous histology.

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HPV PREVALENCE AMONG CLINICALLY HEALTHY ITALIAN MALE POPULATION AND GENOTYPE CONCORDANCE BETWEEN SEXUAL PARTNERS

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Objective: To describe the prevalence and the genotype distribution of HPV DNA among a series of Italian clinically healthy men and the HPV genotype concordance between partners.

Methods: We performed a HPV test on the penile brushings of 378 consecutively selected men (mean age 38 years, range 18-68). The men were stable, monogamous sexual partners of women previously or presently affected by CIN and/or with a positive cervical HPV DNA test. 238 female partners (mean age 36 years, range 18-65) were submitted to a HPV test together with their partners. The amplification and genotyping were performed by Linear Array HPV Genotyping Test (Roche Diagnostics S.p.A).

Conclusions: 153 men (40.5%) and 122 women (51.3%) were HPV positive. Moreover, 34 of the HPV positive men (22%) and 27 of HPV positive women (22%) harboured two genotypes, 24 men (16%) and 11 women (9%) harboured three genotypes, 7 men (4.6%) and 15 women (12%) harboured four genotypes and 4 men (2.6%) and one woman (0.8%) displayed a 5 genotype multiple infection. The most frequent high risk genotype detected in our study populations was HPV 16 (22% in men and 29.5% in women), while the most frequent low risk genotypes were HPV CP6108 (15%) in men and HPV 42 (12%) in women. In 177 out of the 238 couples both partners were HPV status concordant (raw agreement:74.3%, Kappa: 49% (95% CI:[37% - 61%] p-value <0.0001). In 75 couples both partners were HPV positive and in 102 both partners were HPV negative. Moreover, among the 75 HPV positive couples, 51 (68%) had at least one identical HPV genotype. Among these 51 couples, 38 (74%) were concordant for only one genotype, 8 (16%) were concordant for 2 genotypes, 4 (8%) for 3 genotypes and one couple (2%) was 5 genotype concordant. The data analyzed for single genotype showed a significant risk for a partner to be infected with a certain genotype when the other partner is infected by the same genotype, except for type 18. These data could support the hypothesis that male HPV infection is frequent in sexual partners of HPV positive women, and that men could represent an important mean of HPV transmission between sex partners.
THE COST BURDEN OF GENITAL WARTS IN THE UK

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Introduction: Genital warts (GW) are the second most commonly diagnosed sexually transmitted infection in the UK and almost always caused by HPV 6/11. Treatment of GW was estimated to cost £22.4 million in 2003. GW diagnoses have since increased and NHS cost reporting has advanced. Quantifying the current GW cost burden is therefore important.

Objectives: To calculate the total cost burden of GW in the UK.

Methods: An updated economic model was developed utilising GUM expert opinion. 2009 patient GW episodes were obtained from the Health Protection Agency surveillance system and inflated to population estimates for 2010. GP consultations were determined using The Health Improvement Network (THIN) database. Costs were obtained from official sources at 2010 prices.

Results: The model estimated 173,077 GW patients were treated in UK GUM clinics in 2010 (55.6% first episodes, 33.6% recurrent & 10.8% persistent). A total of 49,504 patients were diagnosed by GPs of which 32,722 were GUM referrals. A total cost of £52.4m was estimated (£49.9m from GUM and £2.5m from GP consultations) equating to an average of £276 per treated GW patient.

Conclusions: At approximately £52.4 million, the annual cost of managing GW is substantial. Adopting preventative healthcare strategies, including quadrivalent HPV vaccination, would help to decrease this burden significantly and could confer important resource savings to the NHS.

ABSTRACTS

P 2-1

PRIMARY SCREENING FOR CERVICAL CANCER IN CAMEROON: SELF- VS CLINICIAN-COLLECTED SPECIMENS FOR HPV DETECTION

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Objective: Cervical cancer screening by cytology in low resource setting is difficult to implement due to logistical problem as “speculum collected specimen”. Self-collection of High-Risk Human Papillomavirus (HR-HPV) may become a valuable screening test. Our aim was to determine the agreement between HR-HPV DNA test results on self- vs clinician-collected samples.

Methods: Since July 2009, we initiated a study to evaluate the accuracy of cervical cancer screening in Cameroon based on self-collected HR-HPV DNA testing. Five hundred eighty-two patients aged between 30-65 years old have been included. Women collected a vaginal self-sampling for HPV test using a flocked swab (ESwab, Copan, Brescia, Italy) before undergoing screening with clinician-collected HPV test, visual inspection with acetic acid (VIA) and cytology. The HR-HPV test was done by a Real Time molecular assay (Abbott Real Time High Risk HPV test). Kappa (K) test was used to determine the concordance between self- and clinician-collected HPV samples.

The ESwab samples were successfully analyzed in 98% of the cases. Data were completed for 493 patients. The HR-HPV prevalence was of 14.4% with the following HR-HPV type repartition: 9.8% HPV 16, 11.3% HPV 18 and 78.9% other HR-HPV types. Self-collected HPV tests were positive for 60 patients (12.2%) and 52 (10.5%) for clinician-collected samples. Global concordance was of 93.9% (K=0.7). Abnormal cytology (ASC-US or more) was identified in 6.9% of women. HPV tests were positive in 47.1% of patients with abnormal cytology and in all women with high grade squamous intraepithelial lesions. A 100% concordance was observed between self-and clinician-collected samples in patients with abnormal cytology.

Conclusion: Preliminary data suggest that self-vaginal HPV sampling compares very favorably with clinician cervical HPV sampling and with cytological results.
ANALYSIS OF FOUR-YEAR CERVICAL CANCER SCREENING PROGRAM WITH VISUAL INSPECTION AS PRIMARY SCREENING METHOD IN A SOUTH-WEST RURAL AREA IN CHINA

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Objective: The aim of the study is to evaluate the effect of visual inspection with acetic acid/Logol’s iodine (VIA/VILI) used for screening methods for cervical cancer and pre-cancerous lesions in the rural areas of western China by analyzing the large scale population-based screening data from the demonstration site, that would be help to explore suitable screening methods and obtain experiences which are especially deficient in the rural areas in China.

Methods: 10269 women aged 30-59 years from Fuling County in Chongqing city were recruited from 2006 to 2009. VIA/VILI was the primary screening method followed by colposcopy if the VIA/VILI was positive. Cervical lesions were diagnosed by directed biopsy under the colposcopy. The VIA/VILI negative women or cervical intraepithelial neoplasia 1 (CIN1) were re-screened using the same procedure in the next year.

Conclusions: VIA/VILI can be used as an alternative screening method for cervical cancer and high-grade pre-cancerous lesions among the women aged 30-59 years in China’s rural areas because of its low cost, easy training for the local health providers, and less depending on facilities. One round screening by VIA/VILI can detect more than a half of CIN2, more than four-fifths of CIN3 and almost all the cervical cancer in the population, and the detection rates of CIN2/3 can be increased by two consecutive rounds of screening.

TYPE SPECIFIC DETECTION OF HIGH-RISK HUMAN PapillomaVirus IN SELF-OBTAINED CervicoVaginal SAMPLES APPLIED TO FTA ELUTE CARTRIDGES

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Objectives: Most procedures for self-sampling of cervical cells are based on liquid-based media for transportation and storage. An alternative is to use a dry filter paper media, such as the FTA card or FTA cartridge. Therefore we evaluated if self-sampling of cervicovaginal fluid using a Viba-brush and an indicating FTA elute cartridge™ can be used for reliable HPV typing, when comparing to samples obtained by a physician using a cytobrush and the indicating FTA elute micro card™ and biopsy analysis.

Methods: A total of 50 women with a previous HR-HPV positive test were invited to perform self-sampling using the Viba-brush and the FTA cartridge and thereafter a physician obtained a cervical sample using the cytobrush and a FTA card (1) together with a biopsy for histology and HPV typing. HPV typing was performed using a multiplex real-time PCR assay, HPVir.

Conclusion: All samples contained sufficient amounts of genomic DNA and the self-samples yielded on average 3.5 times more DNA than those obtained by the physician. All women that were positive for HR-HPV in the biopsy sample also typed positive both by self-sampling and physician-obtained sampling. For women with a histological diagnosis of CIN2-3 all three HPV samples showed 100% concordance. A higher number of women were HPV positive by self-sampling than by physician-obtained sampling or by biopsy analysis. The Viba-brush and the FTA cartridge are suitable for self-sampling of vaginal cells and subsequent HR-HPV typing.

COMPARISON OF FIVE CE/IVD LABELED HPV DNA GENOTYPING ASSAYS

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Objectives: To compare results obtained by five commercially available CE/IVD labeled assays for detection of HPV DNA in cervical swabs. Accuracies were determined and clinical samples were investigated. Furthermore, the performance in the routine molecular laboratory was evaluated.

Methods: For evaluation of accuracies, the Quality Control for Molecular Diagnostics (QCMD) 2009 HPV EQA Pilot study panel (www.qcmd.org) consisting of 10 members including one negative sample was used. For the clinical study, 138 cervical swabs with ASCUS+ cytological results were collected in Thin Prep Collection vessels (Cytyc Corporation). Results obtained by the LINEAR ARRAY HPV Genotyping Test (Roche), the COBAS 4800 HPV Test (Roche), the Infinity HPV-QUAD (Autogenomics), the AMPLIQUALITY HPV TYPE (AB/Analytica), and the OPEGEN PapillomaStrip (Operon) were compared. All assays were performed with maximum automation for extraction and hybridization/detection. For the cobas 4800 HPV Test, the cobas x 480 was used for extraction and the cobas z 480 for real-time PCR as recommended by the manufacturer. For the remaining assays, DNA extraction was done on the easyMag instrument (Biomerieux). For PCR, the GeneAmp 9700 (Applied Biosystems) was used. Hybridization and detection was done with the ProfiBlot T48 (Tecan) except for the HPV-QUAD assay which was done on the INFINITI Analyser (Autogenomics). Results were considered as valid if the internal control was detected. The LINEAR ARRAY HPV Genotyping Test was taken as reference method. For the performance study, both overall and hands-on times were compared.

Conclusions: When the results were compared, 63 samples showed identical and 75 discrepant results. Internal controls were detected throughout the entire study. Overall time required for the different assays ranged from 181 to 465 min, manual time from 20 to 70 min. Because of different detection limits, head to head comparison of different assays may not be conclusive. However, assays evaluated in this study showed reliable results regarding their diagnostic capacities. The clinically relevant threshold was yielded by all assays evaluated in this study. When overall time, manual time, and user-friendliness were compared, considerable differences were observed.

PERFORMANCE OF COBAS 4800 HPV TEST COMPARED WITH THE HYBRID CAPTURE 2

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Objective: To evaluate the performance of the cobas 4800 HPV Test in comparison with the HC2 test in women with cytological and histological results. Further, the clinical performance was evaluated using a clinical cut-off of CIN2+.

Methods: The study population comprised 180 archived cervical samples from sexually active women aged 20-65 years old, attending at primary Health-Care Clinics of the National Health Service and Gynaecological Outpatient Clinics that were referred to the National Institute of Health for opportunistic screening and for evaluation of HPV-associated lesions. According to cytology, 33 women had a normal cytology, 45 had ASC-US, 13 had ASC-H, 52 had LSIL, 34 had HSIL, and 3 had SCC. A subset of 160 samples was available for clinical evaluation, based on histological examination of biopsy samples obtained at colposcopy. Of this, 59 were considered to have CIN grade 1 or less (≤CIN1, regarded as controls) and 101 were diagnosed as CIN grade 2 or worse (≥CIN2, regarded as cases). To assess the reproducibility and the cross-reactivity with LR-HPV genotypes, 13 and 8 samples were retested, respectively. Cases that showed discordant results were subjected to a third test, the CLART Human Papillomavirus 2 assay. All analyses were conducted using the SPSS software.

Results: Overall, HR-HPV types were detected in 152 cases (84.4%) with cobas 4800 and in 157 (87.2%) with HC2 test. Analytical and clinical performance of the cobas 4800 Test showed highly comparable outcomes, with very good values of sensitivity (99.0%), PPV, and agreement (k=0.795; concordance level 95.0%) compared with the HC2 assay. The specificity (40.7%) and NPV for ≥CIN2 was higher by the cobas 4800 Test. In addition, the cobas 4800 Test reproducibility was very good (100%), and no cases of cross-reactivity with other LR-HPV genotypes were observed. Discordant results were observed in 9 samples.

Conclusions: The cobas 4800 HPV Test showed an excellent performance for cervical intraepithelial neoplasia grade 2 or worse. The test is efficient, sensitive, reproducible, fully automated, and suitable for large scale testing. Furthermore, this assay has the advantage to concurrently distinguish HPV 16 and 18 from the other HR-HPV genotypes within a single test, which can give more information relative to the predictive value of HR-HPV infection.
IMPROVED PROCEDURES TO RECOVER HPV mRNA FROM BD SUREPATH™ PRESERVATIVE FLUID AND ENHANCE DETECTION WITH THE APTIMA® HPV ASSAY

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Objectives: Messenger RNA (mRNA) purification and amplification from liquid-based cytology media that contains formalin, such as SurePath Preservative Fluid (BD Diagnostics – TriPath; Burlington, North Carolina USA), can be very challenging due to extensive cross-linking, fragmentation and chemical modification of the nucleic acid that progresses over time and at elevated room temperatures. It has previously been reported that the APTIMA HPV (AHPV) Assay can detect 100 fold more HPV mRNA, from high-risk HPV-positive cervical cells (SiHa, ATCC, Manassas, Virginia) in SurePath preservative if specimens are treated for 2 hours at 65°C with proteinase K prior to running the AHPV assay. The objective of this study was to assess alternatives to the proteinase K digestion procedure.

Methods: The AHPV assay is a CE-marked target amplification nucleic acid test for the in vitro qualitative detection of E6/E7 viral mRNA from 14 high-risk HPV types (16/18/31/33/35/39/45/51/52/56/58/59/66/68). mRNA recovery was analyzed under various proteinase K conditions, including different temperatures (room temperature, 37°C and 65°C) and incubation times (1 to 18 hours) and under different specimen storage conditions prior to the proteinase K treatment. SiHa cells (10 to 100,000 cells/reaction) stored in SurePath medium for 3 days at 30°C were used as the model system.

Conclusions: HPV mRNA recovery and detection with the AHPV assay was comparable to the previously published conditions (2 hours at 65°C) when the proteinase K treatment was performed for 1 hour at 37°C or over night at room temperature. Proteinase K spiked into APTIMA transfer tubes was stable for 60 days at room temperature, enabling the user to prepare the tubes in advance. Once an aliquot of the SurePath specimen was transferred into an APTIMA transfer tube, the specimen was stable for 9 months at 4°C. While clinical validation of this procedure is pending, these analytical studies indicate that these alternative treatment options allow for more flexibility for SurePath specimen testing.

CLINICAL PERFORMANCE OF SEVERAL HPV ASSAYS IN WOMEN REFERED FOR HPV TESTING

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Persistent infection by high-risk human papillomavirus (HR-HPV) is a cause of cervical cancer. The use of HPV detection in cervical screening programs may improve the ability to identify women at risk of cervical cancer. Therefore, the development of appropriate methods for the detection of HR-HPV is essential.

Objective: The aim of this study was to evaluate the sensitivity and specificity of several HPV assays using a clinical cut-off of cervical intraepithelial neoplasia grade 2 or worse.

Methods: 778 women participated in the study. All cervical samples were collected in ThinPrep PreservCyt medium during clinical examination for cytological analyses. A subset of 540 samples was available for clinical evaluation, based on histological examination of biopsy samples obtained at colposcopy. Of this, 212 were considered to have CIN grade 1 or less (≤CIN1, regarded as controls) and 328 were diagnosed as CIN grade 2 or worse (≥CIN2, regarded as cases). The evaluation was performed for eight tests: Hybrid Capture 2 (n=529), cobas 4800 HPV (n=200), Abbott RealTime High Risk HPV (n=390), Cervista HPV HR (n=433), APTIMA HPV (n=418), NucliSENS EasyQ HPV (n=418), CLART HPV2 (n=504), and PapilloCheck HPV (n=95).

Results: In 778 women, the HPV positivity ranged between 51.5% (Cervista HPV HR) and 85.3% (PapilloCheck HPV). Compared with HC2, the lowest and the highest agreement value was achieved with NucliSENS EasyQ HPV (κ=0.292; concordance: 76.0±0.8) and Abbott RealTime High Risk HPV (κ=0.893; concordance: 95.5±1.2), respectively. Clinical performance for ≥CIN2 showed a wide range of values for clinical specificity (between 18.5% and 62.5%). All the assays showed a clinical sensitivity higher than 92.0%. Detailed data regarding the assays performances will be presented.

Conclusions: Cobas 4800 HPV, Abbott RealTime High Risk HPV, and APTIMA HPV showed a slightly higher performance than HC2. However, it should be noted that the tests did not all miss the same cases, making comparisons more complex.
EVALUATION OF THE ABBOTT REALTIME HIGH RISK HPV TEST FOR THE DETECTION AND IDENTIFICATION OF HUMAN PAPILLOMAVIRUSES IN ARCHIVAL CERVICAL CANCER SPECIMENS

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Background: The RealTime High Risk HPV test (RealTime; Abbott, Wiesbaden, Germany) is a novel real-time PCR assay based on concurrent individual genotyping for HPV-16 and HPV-18 and pooled detection of 12 HPV genotypes: 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. The assay is validated for use with cervical swab specimens collected in different transport media.

Objective: To evaluate the performance of RealTime for detection of HPV in formalin fixed, paraffin embedded (FFPE) cervical cancer (CC) tissue specimens in comparison to the standard HPV genotyping assay, INNO-LiPA HPV Genotyping Extra CE (INNO-LiPA; Innogenetics, Ghent, Belgium), which allows identification of 28 different HPV genotypes, including all 14 HPVs covered by RealTime.

Material and methods: A total of 62 FFPE tissue specimens obtained from 31 women with histologically confirmed CC and 31 women with histologically confirmed uterine myomas (presumed HPV negative) were included in the study. For each tissue block, three 10 µm thick sections were cut and processed for DNA isolation; the FFPE blocks of CC and uterine myomas were sectioned alternately to control possible sample-to-sample contamination. RealTime and INNO-LiPA were performed as instructed by the manufacturer.

Results: The 136-bp fragment of human beta-globin, serving as an internal control in RealTime, was amplified from all 62 tissue samples included in the study. The 270-bp fragment of human HLA-DP1 gene, serving as an internal control in the INNO-LiPA, was amplified from 20/31 (64.5%) of the CC samples and from all 31 uterine myoma FFPE tissues. HPV-DNA was detected in 30/31 (96.8%) of the CC samples with RealTime, and in none of the uterine myoma specimens. The single RealTime HPV negative CC sample contained HPV-73, which is not targeted by RealTime. Using INNO-LiPA, HPV-DNA was identified in all 31 CC samples and in none of the uterine myoma specimens. There was 100% genotyping agreement between the two methods for 14 HPV genotypes that can be identified by both assays.

Conclusions: The RealTime assay is a reliable, sensitive, and specific diagnostic tool for the detection and partial genotyping of targeted HPV genotypes in FFPE CC tissue specimens.

QUANTIFICATION AND GENOTYPING OF HUMAN PAPILLOMAVIRUS BY REAL-TIME PCR AND HPV DNA CHIP IN CERVICAL SAMPLES

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Cervical cancer is the second-most frequent cancer in women around the world. Infection with high-risk human papillomavirus types (HR-HPV) is closely connected to this cancer. Although Papanicolaou (Pap) smear and Hybrid capture II (HC-II) are commonly used for detection of HPV, these methods have limitation because the correlation between cytology lesion, HPV types and viral loads per cell are not completely reflected.

We introduce improved useful method based on Real-time PCR (RT-PCR) and Microarray HPV genotyping. We tested novel primer sets (GPM7 Forward/Reverse) that target in the conserved L1 region of HPV genome to detect the broad range HPV types, at least 36 types, and evaluation of viral loads per cell. Generated RT-PCR products that are Cy-5 labeled in reverse primers are directly used to screen genotype on microarray.

This assay applied on 150 genital samples that were presented cytological abnormality, and were HC II positive in 64% (n=96) and negative in 36% (n=54). In our results, when RT-PCR negative range was adjusted at below 100 copy, RT-PCR Positive was 80% (n=120) and negative was 20% (n=30). Genotyping was sequentially performed with RT-PCR Positive samples by microarray. 85.5% of 55 ASC-US (Atypical Squamous Cells of Undetermined Significance) classified samples were identified genotype, mainly type 16 (16.4%), and 14.5% of them were negative. Each HPV positive ratio was 85.5%, 86.7%, 96.9% and 100% in ASC-US, LSIL, HSIL and Cancer.

Although the relation of statistical significance between viral load and cytology was not cleared, we verified its increased pattern in high grad lesion. We will study with more clinical samples for precise statistical significance test. Quantification and identification of HPV by connected methods with RT-PCR and DNA chip will be helpful to predict the progression of cervical cancer.
A NOVEL, AUTOMATED DNA EXTRACTION METHOD FROM CERVICAL SUREPATH® SPECIMENS


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**Objectives:** The *digene* HC2 High-Risk HPV DNA Test® (HC2 test) is validated for use in Europe with cervical specimens collected in SurePath cytology media. We developed a new automated protocol (based on the QIAsymphony AXpH chemistry) to purify DNA from cervical SurePath® specimens. In our initial R&D study presented here we investigated the performance of this protocol using a) individual SurePath specimens and b) cell culture samples in SurePath medium spiked with potentially interfering substances for subsequent downstream use with the HC2 test. The QIAsymphony applications presented here are for research purposes. Not for use in diagnostic procedures.

**Methods:** a) Individual SurePath specimens: To compare manual and automated sample preparation methods, DNA was isolated on the QIAsymphony using the newly developed AXpH SurePath protocol, which included a Proteinase K digest with extended lysis and as a reference, the established manual sample conversion method. The QIAsymphony eluates and the corresponding manually converted pellets were tested with HC2. Testing was done using 144 residual, de-identified, clinical SurePath samples. Cervical SurePath post-gradient specimens, retained after cytology screening, were used.

b) Interfering substances: The impact of potentially interfering substances on the AXpH chemistry were tested by adding varying amounts of blood, lubricating jelly, contraceptive jelly, spermicidal gel, douche, feminine spray, and antifungal cream to low positive cell culture samples.

**Results:** a) Individual SurePath specimens: The QIAsymphony AXpH SurePath protocol resulted in 96% total agreement with the manual conversion method (138 of the 144 results agreed).

b) Interfering substances: Of the 7 potentially interfering substances, no significant impact on the performance of the SurePath AXpH protocol has been observed. Mean RLU/co values of all low positive samples stayed above the cut-off (>1).

**Conclusion:** The newly developed QIAsymphony AXpH SurePath protocol demonstrates the feasibility of DNA extraction from cervical SurePath specimens for use in hybrid capture based downstream applications. The performance of the automated DNA extraction method is not compromised by the presence of the potentially inhibitory substances at the tested concentrations.

COMPARATIVE STUDY OF HPV GENOTYPES AND CLINICAL DIAGNOSIS OF CARCINOMA OF THE ORAL CAVITY THROUGH REVERSE HYBRIDIZATION

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Squamous cell carcinomas (SCCs) represents the most frequent malignancy in the head and neck region (1). The number of newly reported cases is estimated to be around 500,000 per year, worldwide. Common risk factors in head and neck squamous cell carcinoma are smoking and alcohol abuse (2). In recent years, a role of human papillomavirus (HPV), in the 70% of the head and neck cancer was postulated, making HPV 16 the most prevalent type in oral cancer (3).

**Objective:** Perform a comparative study between the HPV type detected and the clinical diagnosis of patients with carcinoma of oral cavity, relating to demographic factors (age and gender) and smoking habits and alcohol.

**Methods:** Specimen adequacy was evaluated by examining slides stained with hematoxylin-eosin for each case to determine the diagnosis. DNA from 24 paraffin-embedded tumor sections of oral cavities was extracted with the QIAGEN kit (Hilden, Germany) and the detection and typing of HPV was performed by reverse hybridization by the INNO-LIPA HPV Genotyping Extra kit (Technologiekpark Innogenetics NV Belgium).

**Results:** The study included 21 squamous cell carcinoma and 3 epidermoid carcinoma, of which 79.16% were males, with an average age of 55.95 years and 20.83% females, with an average age of 73.4 years. In the squamous cell carcinomas specimen 76.19% of positivity was found; of which 37.5% were well differentiated squamous cell carcinomas, 50% moderately differentiated squamous cell carcinomas and 6.25% poorly differentiated squamous cell carcinomas and invasive squamous carcinomas, respectively. The most common genotype was 16, followed by types 6 and/or mixed infections with types 11, 18, 31, 39, 44, 53 and 56.

In the epidermoid carcinomas 66.67% of positive samples was found; of which 33.33% were well differentiated squamous cell carcinomas and 66.67% moderately differentiated squamous cell carcinomas, detecting HPV types 6 and 11, respectively.

**Conclusion:** We found a 75% positivity for the total sample. Type 16 was the most frequently found in 66.67%, either alone or in mixed infections. In Venezuela, the head and neck cancer that is associated with HPV infections is a major risk factor in adults.


COMPARISON OF THE NEW COBAS® 4800 HPV TEST AND A CONSENSUS HPV PCR/SEQUENCING ASSAY COMPLETED WITH INNOLIPA GENOTYPING

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Objectives: Carcinogenic HPV DNA testing will soon be widely available as a primary screening method to prevent cervical cancer. Various high-risk HPV (HR-HPV) detection methods exist but there is the need for a reliable automated robotic system, for high throughput HPV testing. We evaluated concordance between our routinely HPV testing method consisting in the MY09/MY11 consensus PCR screening method following by DNA sequencing and/or iNNOLIPA genotyping assay and a fully automated assay corresponding in the COBAS® 4800 HPV test which detects a pool of 12 HR-HPV (genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in aggregate with concurrent, separate detection of HPV16 and HPV18.

Methods and results: A total of 154 cervical samples, performed during routine gynaecologic follow-up, were tested. Concordance between the two methods was calculated using kappa analyses in the limitation to those HR-HPV detected by both genotyping tests. The overall agreement between the two methods for the presence or absence of HR-HPV including genotypes 16 and 18 was 89.6%, kappa 0.73. The concordances for the presence or absence of HPV16, HPV18 and HPV-HR were 146/154 (95%, kappa 0.87), 146/154 (95%, kappa 0.74) and 134/154 (87%, kappa 0.84) respectively.

Conclusions: The COBAS® 4800 HPV test appears to be an accurate and sensitive method for detection and genotyping HR-HPV infection. Furthermore, this test is a highly automated system from the extraction to the genotyping step, allowing results in less than 4 hours for 94 samples. This test is therefore well adapted for a high throughput HR-HPV based cervical screening tool.

DEVELOPMENT OF AN HPV GENOTYPING ASSAY CAPABLE OF SPECIFICALLY DETECTING HPV 16, 18, AND 45

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Objectives: The digene HPV Genotyping PS Test (PS) RUO was developed for the specific detection of HPV 16, 18, and 45. To assess the performance of the PS Test cervical clinical specimens collected in both QIAGEN Specimen Transport Media (STM) and Hologic PreservCyt® media (PC) was tested by four independent labs. Accuracy of the PS Test was determined by comparing the results to a QIAGEN in-house validated sequence specific qPCR method for the detection of HPV 16, 18, and 45.

Methods: The PS Test is a non target amplification assay based on Hybrid Capture® technology. The test utilizes QIAGEN’s proprietary hybrid-specific antibodies for the detection of HPV DNA targets. The specific detection of HPV 16, 18, and 45 is performed in separate wells using RNA probe cocktails custom tailored for optimal specificity against all other HR- and LR-HPV genotypes. The sample volume inputs for detection of each of the three HPV types (16, 18, and 45) are equal to the sample volume input of the HC2-HR screening assay, which is 75ul of denatured STM or 2ml of undenatured PC. Performance of the PS Test was compared to type specific quantitative-PCR (qPCR) to evaluate concordance. For the qPCR test, DNA was extracted from clinical specimens using the QIAamp® Media kit (QIAGEN) and standard curves were generated using serial dilutions of HPV 16, 18, or 45 plasmid at 10E+00 to 10E+07 copies/assay. All qPCR assays were executed on the Rotor-Gene® Q real time PCR thermal cycler system (QIAGEN) using the QuantiTect® Virus Kit (QIAGEN). For each of the three HPV types of interest, primers and probes were designed within the E6/E7 region of the HPV genome.

Conclusions: By using sequence specific qPCR as our reference method, we were able to demonstrate the accuracy of the digene HPV Genotyping PS Test (PS) in detecting HPV 16, 18, and 45 at the clinically significant cut-off of 5000 copies per assay. Over 450 total STM and PC samples were tested with PS by four independent labs and qPCR in-house. The overall concordance between the two detection methods was shown to be greater than 90%. The applications presented here are for research use only and are not to be used for diagnostic procedures.
PREVALENCE OF HIGH-RISK HUMAN PAPILLOMAVIRUS IN HIGH GRADE LESION OF CERVIX

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Cervical cancer is the second most common cancer in Thai women and highly mortality rate from disease. High grade lesion of cervix is precancerous lesion. Human papillomavirus (HPV) is major course of this disease.

Propose: To identified prevalence of high-risk HPV infection in cervical high grade lesion.

Methods: This is cross-sectional study design. Three hundred and fifty five specimen of cervical high grade lesion was tested by Innogenic-Line-Probe-Assay (INNO-LiPA). The INNO-LiPA is the newest HPV test in Thailand and can detected multiple type infection of HPV.

Result: Overall high risk HPV infection was found about eighty percents of high grade lesion by INNO-LiPA. The most common type in high grade cervical lesion is 16, 18, 31, 33, 52, 58. This test can detect multiple type of high-risk HPV infection in specimen. The multiple types were found about ten percent of high grade cervical lesion. The most HPV type in multiple infections is 16 and 18.

Conclusions: The prevalence of high-risk HPV infection in cervical high grade lesion of Thai women was very high, is higher than WHO 2007 report. The most cases found HPV type 16 or 18 and some cases found multiple type infection of HPV.

CORRELATION BETWEEN IMMUNOHISTOCHEMISTRY OF HPV L1 CAPSID PROTEIN AND BEHAVIOR OF LOW-GRADE CERVICAL CYTOLOGY IN KOREAN WOMEN

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Objectives: The aim of this study was to evaluate the behavior of LSIL in Korean women infected with HPV in relation to the immunocytochemical detection of the HPV L1 capsid protein.

Methods: From January 2006 to December 2007, a total of 353 immunocytochemistry were performed on specimens from HPV-infected patients with LSIL. Due to exclusions, the study population was reduced to 318. Subjects were monitored at 4–6 month intervals. The regression, persistence, and progression of the cytologic abnormalities of the 318 cases were compared with the results of HPV L1 capsid protein immunocytochemical detection.

Conclusions: Of the 137 patients negative for the HPV L1 capsid protein, 38 (27.7%) showed progression to high-grade lesions, 50 (36.5%) showed persistence, and 49 (35.8%) showed regression to normal cytological features. In contrast, of the remaining 181 patients positive for the HPV L1 capsid protein, 15 (8.3%) showed progression to high grade lesions, 74 (40.9%) showed persistence, and 92 (50.8%) showed regression. The results of immunocytochemical testing for the HPV L1 capsid protein show a linear association with the progression or regression behavior of low-grade cervical cytology in patients infected with HPV (linear by linear association test, \( P < 0.05 \)). In conclusion, immunocytochemical detection of HPV L1 was significantly related with the biological patterns of LSIL in Korean women. Hence, immunocytochemistry for the detection of HPV L1 is beneficial in providing further information for LSIL.
A CASE-CONTROL STUDY OF THE KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) GENES AND GENOTYPES IN RELATION TO HPV-MEDIATED CERVICAL NEOPLASIA IN KOREAN WOMEN

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Objective: Infection with high-risk human papillomavirus (HPV) is the major risk factor to develop cervical neoplasia. However, host and viral genetic factors are likely to play a role in this process, along with environmental and lifestyle factors. NK cells play a pivotal role to protect from viral infections and the early development of cancers. Killer cell immunoglobulin-like receptors (KIR) are a kind of receptors which are expressed on NK cells and it bind to some HLA class I alleles. Six activating KIR genes and 9 inhibiting KIR genes are encoded on chromosome 19q13.4. In this study, we intended to investigate the influence of the polymorphism of the KIR genes and genotypes on genetic susceptibility in human papillomavirus infections in Korean women.

Methods: A total of 153 Korean women with cervical neoplasia associated with human papillomavirus infection who presented to the department of obstetrics and gynecology at Seoul St. Mary’s Hospital from December 2010 through December 2011 were selected for this study. These patients were compared to 100 controls randomly selected during the study period with benign gynecologic diseases such as myoma of uterus, ovarian cyst, and uterine prolapse. KIR genotypes and were determined using PCR-SSP method.

Conclusion: The frequencies of KIR3DS1 were significantly decreased in cervical neoplasia patients compared with normal controls (OR 0.49, p=0.03). These results suggest that the KIR3DS1 gene and genotype associated with function of NK cells may affect on the occurrence of cervical neoplasia.

CLINICAL AND MOLECULAR CHARACTERIZATION OF SMALL VULVAR CANCER OF THE ANTERIOR FOURCHETTE

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Background: The incidence of vulvar cancer in women has increased in Germany during the last decade. The mean age has dropped by about 8 years in our own study group and we observed a significant increase of small tumors located between clitoris and urethra.

Objective: We initiated a multicenter study in Germany asking members of the Study Group for Colposcopy and gynecological centers specialized in the treatment of vulvar cancer to provide tumor samples from patients with invasive tumors (max. T2) strongly located between clitoris and urethra.

Material and Methods: We are analyzing 138 tumor samples from vulvar cancer of the anterior fourchette and 54 vulvar cancer located at different sites of the vulva. We have access to clinical and histological data, treatment characteristics and anamnestic data from most of the patients. We will analyze these tumor samples and control samples for p53 and p16 INK4a expression by IHC and the presence of HPV DNA and if positive HPV-type.

Results: The tumors located between clitoris and urethra show more frequently strong p53 expression than tumors from other vulvar sites (39.7% vs. 27.5%) and less frequently diffuse p16(INK4a) overexpression (29.4% vs. 35.0%). p53 and p16(INK4a) expression in tumors between clitoris and urethra show inverse correlation (p=0.003). Ki-67 expression in 70% or more of the tumor cells was found in similar frequencies at the different localizations (27.5% vs. 26.6%). Data on the presence of HPV DNA and HPV-types in the tumor samples are preliminary and will be presented if verified.

Conclusion: Our results suggest that particularly in vulvar cancer located between the clitoris and urethra there are more often tumors in which p53 seems to play a role as key tumor suppressor gene in tumorigenesis, as overexpression of p53 is known to be associated with p53 gene alterations. A molecularly different entity of vulvar cancers shows p16(INK4a) overexpression and these tumors appear to be more frequent at localizations other than between clitoris and urethra. p16(INK4a) overexpression is known as a marker for transforming high risk human papillomavirus (HPV) infections and genotyping of HPV in the analyzed vulvar cancers is currently completed. The results will help to develop specific diagnostic and therapeutic strategies in the future for the apparently heterogenic tumor entity of vulvar cancers.

ABSTRACTS
PROFILING OF LOW ABUNDANT PLASMA PROTEOME OF CERVICAL CANCER BY TWO DIMENSIONAL LIQUID PHASE CHROMATOGRAPHY

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Objective: Cervical cancer is one of the high morbidity disease in Uyghur women?490?526/100,000?and the mortality rate is up to four times higher than the other ethnic groups living in the same region of Xinjiang province, China. The aim of this study was to establish a plasma proteome profile for the screening of protein markers specific to Uyghur women with cervical cancer.

Methods: Plasma samples were collected from Uyghur women with cervical diseases including cervical squamous cell carcinoma (CSSC, n=26), cervical intraepithelial neoplasia (CIN) II/III (n=21) and chronic cervicitis as a control (n=22). After depletion of high abundant plasma proteins, the low abundant plasma proteome was separated and analyzed by 2-D liquid phase chromatography (ProteomeLab™ PF-2D).

Results: Based on the protein isoelectric point gradient and hydrophobic features, unique proteome fingerprints was established for different groups of patients including CSCC, CIN and cervicitis. By setting up at least two fold alteration of protein content between two groups compared at corresponding peak constituents as a cutoff value, we found that 9 peak constituents were upregulated and 6 downregulated in patients with CSCC in comparison to chronic cervicitis, whereas 10 peak constituents were upregulated and 4 downregulated compared to CIN II/III. In case of CIN II/III, 5 peak constituents were upregulated and 5 downregulated in comparison to the chronic cervicitis.

Conclusion: This proteomic approach with two-dimensional liquid phase chromatography provided valuable data about the unique fingerprint features of low abundant plasma proteome specific to Uyghur women with cervical cancer or its precursors, and may lead to the identification of plasma protein markers of the cancer in mass spectrometry based studies in the future.

This work was supported by High Technology Research and Development Fund of Xinjiang(200910106)and Nature Science Fund of Xinjiang(2010211B15).

ROLE OF P16INK4a, AND hTERT IN EARLY DETECTION OF CERVIX CANCER

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Due to cancer’s several faces, it is hard to identify a single marker aimed to discover correctly early stage transformation. In this context, we studied p16INK4a and hTERT clinical utility as diagnosis markers, as well as their role in early detection of disease in preclinical stages.

Methods: Cervical smears obtained from 50 women with/without suggestive HPV infection pathology were cytological investigated. HPV DNA testing was done using IINNOLIPA kit. Semi-quantitative expression levels of p16 INK4a and hTERT were estimated in RT-PCR.

Results: p16INK4a and hTERT expression levels were correlated with cytological degree of cervical lesions. p16INK4a values were 1.36 times greater in LSIL patients than in NILM subjects (p = 0.07), and 2.38 times greater in HSIL/cancer patients as compared with NILM patients (p = 0.002). Significant differences in p16 expression between ASCUS: HSIL group (p = 0.02) and LSIL: HSIL (p = 0.07) group was observed. p16INK4a expression level was correlated with the presence of hrHPV in low and high risk lesions. On the other hand, hTERT mRNA expression was significantly increased in LSIL (p = 0.035) and HSIL/cancer (p=0.0044) versus normal group. hTERT expression in ASCUS patients has no statistical significance as compared with the normal group (p=0.37). In single high-risk genotype infections the correlation p16:hTERT is very increased r=0.63 while in co-infections, an inverse correlations occurs, p16:hTERT r=-0.02. Positive correlations were noticed in HSIL/cancer group for p16:hTERT.

Conclusions: We consider that p16INK4a and hTERT are of real interest as tumor markers for gynecological oncology

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EVALUATION OF MCM-2 AND P16 IN CERVICAL CANCER TISSUE MICRO-ARRAY (TMA)
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Background: Efforts have been undertaken to identify reliable biomarkers for cervical cancer screening programs. Minichromosome maintenance proteins (MCM) and p16INK4a have emerged as promising proliferation markers in several tumor types (1,2).

Aim: We examined cervical cancers for these proteins to determine if either may be a biomarker of cervical cancer.

Materials and Methods: By means of immunohistochemistry, a total of 488 Tissue Micro-Array (TMA) formalin-fixed, paraffin-embedded cervical cores were analyzed for MCM-2 and p16INK4a. 308 samples were invasive cervical cancer (SCC/Adeno) and 80 normal cervical epithelium cores were used as controls. In situ hybridization was used to detect HPV DNA. Statistical analysis was carried out using STATA 10.0 and logistic regression analysis was done for multiple comparisons.

Results: Expression was significantly increased in invasive cervical cancer cases versus controls for both MCM-2 (Odds Ratio (OR)= 5.15; 95% Confidence Interval (CI): 2.67–9.93) and p16 (OR= 1.13; 95% CI: 1.09–1.17). Controls (mean=39 years) were younger compared to the invasive cervical cancer cases (mean=47 years) respectively. HPV DNA was found in all invasive cervical patients.

Conclusions: MCM-2 and p16 were highly expressed in the invasive cervical cancer compared to controls, though, additional studies with different CIN grade need to be carried out to evaluate their potential utility as prognostic CIN biomarkers.

References:

P16INK4A/Ki-67 DUAL STAIN CYTOLOGY IN SAMPLES FROM KENYAN WOMEN
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Objectives: The prevalence of high risk human papillomavirus (HR-HPV) infection and related diseases in Kenya is high. Limited infrastructure warrants the evaluation of markers that specifically detect high grade cervical lesions which need treatment, in order to avoid repeated testing or intensive work-up. We evaluated the applicability and performance of p16INK4A/Ki-67 dual-stain cytology in a screening population in Kenya and compared it to the Hybrid Capture 2 HR-HPV DNA test.

Methods: A total of 498 PreservCyt samples were collected from women without known history of cervical dysplasia, cancer or HPV infection in Thika district, Kenya. ThinPrep Pap cytology (Hologic), Hybrid Capture 2 HR-HPV test (Qiagen) and p16INK4A/Ki-67 dual-stain cytology (CINtec PLUS, mtm Laboratories) was performed.

Conclusions: Valid results for all tests could be obtained in 485 samples. HR-HPV DNA by HC2 was detected in 104/485 (21.4%) of the samples. Most women had normal cytology (431, 88.9%), 14 (2.9%) had LSIL, 1 (0.2%) AGUS, 28 (5.8%) ASCUS and 11 (2.3%) had HSIL. p16INK4A/Ki-67 cytology was positive in 39/485 (8.0%) of the samples. HC2 was positive in 9/11 (81.8%) of HSIL and p16INK4A/Ki-67 was positive in 8/11 (72.7%) of HSIL samples. In samples diagnosed as normal in Pap cytology, the rate of positive HC2 results was with 77/431 (17.9%) substantially higher than the rate of positive p16INK4A/Ki-67 samples (15/431, 3.5%). As the situation at the study site precluded systematic colposcopy and biopsy follow-up, no histology is available for most of the women and thus diagnostic sensitivity and specificity of p16INK4A/Ki-67 dual-stain cytology in Kenyan women remains to be adequately evaluated. However, our study is the first demonstrating the general technical applicability of p16INK4A/Ki-67 cytology in a developing country screening population. Keeping in mind the limited reliability of Pap cytology as disease indicator, our finding points to a lower fraction of false positive test results using p16INK4A/Ki-67 cytology compared to HC2, which would be important for a point-of-care test in limited recourse settings.

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NEGATIVE CORRELATION BETWEEN DYNAMIN 2 EXPRESSION AND DEGREE OF CERVICAL INTRAEPITHELIAL NEOPLASIA: A COMPARISON WITH KI-67 EXPRESSION AND DETECTION OF HPV TYPES

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Objectives: Dynamin 2 is known as a GTPases which is associated with extracellular matrix remodeling and endocytosis. Recent studies suggested that human papillomavirus (HPV) could enter into host cells by dynamin 2-related endocytosis. In this study, we investigated the expression of dynamin 2 protein in cervical intraepithelial lesions (CIN) by comparing with Ki-67 expression and type of HPV infection (low risk vs. high risk).

Methods: Biopsy samples (n= 66: reactive changes, 7; CIN I, 33; CIN II, 14; CIN III, 12) were analyzed by immunohistochemistry for expression of dynamin 2 and Ki-67 as well as by using Oligonucleotide DNA Chip for HPV detection. Degree of dynamin 2 and Ki-67 expression was shown to have negative and positive correlation with degree of CIN, respectively (P < .001 and P < .001). However, there was no relationship between dynamin 2 or Ki-67 and type of HPV infection. Dynamin 2 was not expressed in all of CIN II/III lesions except one case in CIN II (25/26, 96.2%). Concerning the sensitivity for detecting CIN II/III, negative expression of dynamin 2 is more sensitive than high expression of Ki-67 (96.2% vs. 73.1%, P= .041).

Conclusions: These results suggested that dynamin 2 may be helpful biomarker for grading CIN lesions with high sensitivity in diagnose of high grade lesions (CIN II and III) when the expression of dynamin 2 was negative. To our best knowledge, this is the first study dealing with dynamin 2 and cervical dysplasia, especially in grading CIN lesions.

PRETREATMENT NEUTROPHIL-LYMPHOCYTE RATIO AS A PROGNOSTIC FACTOR IN CERVICAL CARCINOMA

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Objectives: Immunologic responses including pretreatment neutrophil and lymphocyte counts are associated with survival in many cancers. This study evaluated the prognostic value of the neutrophil-lymphocyte ratio (NLR) in patients with cervical cancer.

Methods: Patients with clinically staged cervical carcinoma (IB to IVA) at Samsung Medical Center, Seoul, Korea, from 1996 to 2007 were retrospectively enrolled. We enrolled 1061 patients with cervical cancer. The median value of NLR was 1.9 with the range of 0.3-27.0. When the cohort was divided according to this median value of NLR, poorer survival outcomes were observed in higher LNR group (≥1.9) than in lower NLR group (<1.9). Higher NLR group (≥1.9) was younger in age and had more advanced staged disease when compared with those of lower NLR group (<1.9). In multivariable analysis, pretreatment NLR was identified as independent risk factor for survival, which showed that higher NLR entailed poorer survival.

Conclusions: Pretreatment NLR was an independent predictor of survival in cervical cancer patients even after adjusting age, stage, cell type, and type of primary treatment and may be a cost-effective biomarker to stratify risk of recurrence and death in cervical cancer patients as well as clinical stage.
As the majority of cervical cancers are associated with HPV genotypes from two distinct Alpha-Papillomavirus clades A7 (HPV18, 39, 45, 59, 68) and A9 (HPV16, 31, 33, 35, 52, 58), the extent to which HPV16/18 vaccines will protect against related genotypes is an important unresolved issue. Few published data are available on the frequency or titre of neutralising antibodies against closely-related, non-vaccine types.

**Objectives:** To determine the frequency and titre of neutralising antibodies against a range of A7 and A9 HPV types in sera from individuals immunised with CervarixTM within the UK National HPV Immunisation Programme.

**Methods:** Serum samples were collected from 69 girls aged 13-14y, a median 5.9 months (IQR 5.7-6.0) after their third CervarixTM vaccine dose. Neutralisation assays were performed using L1L2 pseudoviruses from a range of A7 and A9 HPV types and control Bovine Papillomavirus.

**Results:** Cross-neutralising antibodies against HPV31 were common (96% of sera). Reactivity against other A9 types was less common (36 – 67% of sera depending on the HPV type). Cross-neutralisation titres for non-vaccine types were substantially lower than for vaccine types; for example, the geometric mean titres (GMT) for HPV31 was 0.4% (95% CI, 0.31 – 0.51%) of the HPV16 titre, with the GMT for other A9 HPV types ranging from 0.06 – 0.10% of the HPV16 titre. Data for the A7 genotypes will be presented. The specificity of these responses was assessed using VLP ELISA, total IgG and anti-VLP depletion assays and vaccine-naïve sera. The low prevalence of these HPV types in the population and the ages in the study cohort, suggest these responses are due to vaccination.

**Conclusions:** Here we show that neutralising antibody responses against closely-related, non-vaccine types are common, but the titres are very low (≤ 1% of type-specific titre). Studies have shown that HPV16/18 antibody titres in genital secretions are much lower than those found in the periphery. It is unclear whether these low levels of antibodies would be sufficient to protect against infection in the absence of other immune mechanisms. Their utility as surrogate markers of protection remains to be determined.
CIN 3 TREATMENT AND ITS CONSEQUENCES ON FURTHER PREGNANCIES

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Objective: The aim of our study was to assess the outcome of pregnancy in the fertile women treated for CIN 3.

Material and method:
- We carried out a study over 77 women who had undergone treatment for CIN3 between 2005-2009.
- Among this group, 53 patients were classified in the fertile group (19-41 years).
- 16 patients were primiparous or secoundiparous and 37 were nulliparous women.
- All 53 patients had at least one pregnancy after the cone biopsy treatment.
- This review encompasses the diagnosis of lesions, therapeutic management and the follow up of the women.
- Diagnosis was made through Pap-smear which were classified according to the Bethesda system, colposcopy, viral identification of the HPV types, histopathological results and immunohistochemical staining.
- Pap smear exam demonstrated ASC-US, ASC-H, LSIL, HSIL in 85%.
- HPV determination was performed in all 53 cases. The most frequent HPV type was HPV 16 in 27 cases followed by HPV 31, 53, 51, 18, 66, 45.
- The treatment performed was cone biopsy for all 53 cases, follow up was done at 6, 12, 18, 24, 36 months and in 8 cases LLETZ was performed for recurrence.
- As obstetrical consequences for the aforementioned group we can specify:
  - spontaneous abortions 9 cases (1 twin pregnancy) -17%
  - premature birth 20 cases -37%
  - term birth 24 cases -46%

Conclusions:
1) Regarding the high number of prematur births and spontan abortions which can be caused by cone biopsy or LLETZ we recommend for fertile women very strict criteria for the diagnosis of CIN 3, which should include pap smear, colposcopy, identification of the HPV types, histopathological diagnosis and immunohistochemical staining.
2) Women with cervical lesion particularly with CIN 3, who wish a further pregnancy, should be properly informed about all the risk factors and consequences of the surgical treatment of CIN 3, which for many women seems harmless.

CERVICAL INTRAEPITHELIAL NEOPLASIA IN YOUNG WOMEN

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Introduction: Cervical cytology screening programs have clearly led to the decline of cervical cancer in developed countries. Several authors have shown that the risk of high-grade cervical intraepithelial neoplasia (CIN) is substantial among females with abnormal cervical cytology, ranging from 8% to 50%.

Most guidelines were overaggressive in their management of abnormal cytology in adolescents and young women. The purpose was study young women (under 30 years old) who had undergone a loop electrosurgical excision procedure (LEEP) or laser conization procedure.

Methods: Retrospective analysis of a retrospective database (4 years) of young women (< 31 years) undergoing conization procedure in CHTS, Penafiel. We created two groups the group 1 young women with low-grade CIN and the group 2 young women with high-grade CIN in conization biopsy results.

Results: Among 59 eligible young women, low-grade CIN were found in 32.3% and high grade CIN in 67.7%. on histology of LEEP/ laser conization procedure.

No differences were found in age, parity, and history of other sexually transmitted infections, smoking, oral contraceptive, condom use, age at first intercourse and the number of years since first intercourse among two groups.

On colposcopic histology in both groups the concordance with conization histology was high (k = 0.67).

When we compared the referral Pap with conization histology the concordance in group 2 was lower.

Conclusions: In our study 37% of young women with LSIL in cytology have high grade CIN.

These results conflict with current recommendations for less aggressive follow-up for most young women with low-grade CIN.
EFFECTIVENESS OF CONIZATION ON TREATING CIN LESIONS – 5 YEARS RETROSPECTIVE STUDY AT THE HOSPITAL OF FARO - EPE (PORTUGAL)

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Responsible for the Colposcopy Unit: Pacheco, A
Responsible for the Anatomy and Pathology Department: Enriquez, J
Responsible for the Gynecology and Obstetrics Department: Viseu, O

Objectives: CIN lesions, as precursors for cervical cancer, are of major importance and should carefully be observed, followed and treated. Different treatments might be used: ablation or excision, having different future outcomes, especially in terms of reproductive life. In our department we only have available excisional treatment, namely cold-Knife conization and loop excision. As that, we would like to evaluate if our resources and how they are used, are effective enough on treating CIN lesions.

Methods: It was done a 5 year (2006-2010) retrospective study of all women followed in our Colposcopy Unit and that were submitted to conization. Some of the variables taken in count were: age; parity; risk behaviors; contraceptives used; motive of referral to the unit; Pap smear alterations; biopsy histology; type of conization; margins; HPV testing and follow up. During these years it was done 6730 colposcopies and more than 1000 biopsies; around half of these were conizations. Seldom it was needed a new conization and the margins were mostly free of disease.

Conclusion: The main objective in the follow-up of patients after conization is the early detection of residual or recurrent cervical disease. An optimal method of follow-up after conization is therefore crucial. It is very important to review/audit the procedures and outcomes of every colposcopy unit in order to implement validated protocols, adjusted to each population. Our Unit has been growing in the last 5 years, having more professionals and more differentiated. Cold-knife conization was more used in the early years and with practice, loop in gaining relevance. It would be useful if ablation treatments were available. Even though some limitations, our effectiveness is quite high, with few margins affected.

WOMEN UNDER 30 WITH HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESIONS – 2007 TO 2010: OUR EXPERIENCE

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Objectives: Despite the introduction of cervical screening programs, the incidence of cervical cancer in Portugal is still high (13.5/100,000) and responsible for 4.2% of malignancy-related deaths among women. Organized screening programs aside, global sensitivity rates of cytology are 68% (conventional) and 76% (ThinPrep), and specificity rates are 79% (conventional) and 86% (ThinPrep). HPV testing has greater sensitivity and reliability than cytologic screening for detection of cervical pre-cancer (cervical intraepithelial neoplasia grade 3 (CIN3)) and cervical cancer (>CIN3). Nevertheless, the use of HPV testing in primary screening is only recommended for women aged 30 years or older because these women are typically past the peak age of self-limited infections, frequent in a younger age group. Thus, the positive predictive value of ?CIN3 is higher in women aged 30 years. NCCN guidelines recommend a loop electrosurgical excision procedure (LEEP) in the treatment of high grade lesions in adults older than 21 years. Concerns regarding future pregnancy outcomes rise in young women submitted to LEEP.

Methods: The authors reviewed the cases of women under 30 years, submitted to LEEP due to a diagnosis of CIN3 from 2007 to 2010. The variables analyzed were age, risk factors, follow-up and outcome.

Conclusions: Fifteen women underwent LEEP. The average age was 25 years. Estroprogestatives were contraception method used in 67% and 13% of patients were smokers. The average age of first sexual intercourse was 17.6 years. The most frequent reasons for referral were HSIL and LSIL on cytological screening. Three women underwent a second LEEP. Currently 13 patients were considered cured and 3 of them became pregnant since then, with favorable outcomes. Albeit uncommon, when CIN3 lesions occur in women younger than 30 years, some discomfort arises regarding treatment. LEEP is associated with an increased risk for overall preterm delivery, preterm delivery and low birth weight infants in subsequent pregnancies. Patients considering future pregnancies should be aware of these risks. Although the number of patients is small and the follow-up period is short, this study revealed no significant influence on the obstetric outcome in patients treated for precancerous lesions through the LEEP.
**HSIL: CORRELATION BETWEEN CYTOLOGY, COLPOSCOPY AND HISTOLOGY**

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**Introduction:** Cervical cancer has remained one of the main oncologic causes of death in young women. Several studies show that the introduction of cervical cytology screening programs reduced the incidence of cervical cancer and mortality from this disease.

This reduction is essentially due to the identification and active management of patients with cancer precursors or early invasive cervical cancer. These cancer precursors found by cytology have been divided in the Bethesda System in low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL). This last cytology finding is present in 70% of women with CIN 2/3 and 1 to 2% with invasive cancer. Nonetheless, for the diagnosis of cancer, cytology alone is not sufficient. Colposcopy and histopathology are complementary and essential for a correct evaluation.

**Objective:** Determine the correlation between colposcopic and histopathological findings in women with a HSIL result in cytology.

**Methods:** Revision of the histopathological and colposcopic findings in women observed in our colpocopy unit between January 2006 and December 2010.

**Results:** In the period studied our unit performed a total of 6730 of colposcopies. HSIL was the indication for colposcopy in 177 women. The colposcopy and histopathological findings will be presented later.

**Conclusion:** The presence of an advanced colposcopic unit in our hospital allowed us to review the cytologic indications for colposcopy and relate them with other findings. This is particularly important to evaluate our work during the last 5 years, since we are the reference center in our region for cervix pathology.

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**OPTOELECTRONIC SCANNER TRUSCREEN IN DIAGNOSTICS OF CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS**

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**Objectives:** evaluation of efficacy of real-time optoelectronic scanner TruScreen in diagnostics of cervical intraepithelial lesions.

**Patients and methods:** Participants: group of 102 volunteers aged from 18 to 56 y.o. recruited by FS SCOGP during May 2009.

**Methods:** primary observation with optoelectronic scanner TruScreen (produced by Polartecnics ltd.) followed by conventional methods (PAP test, colposcopy and biopsy/histology).

If the results after TruScreen, cytology and colposcopy were all negative then the histological examination was not performed. If any of the results were positive then the histological examination was performed. The Bethesda system was used in diagnosis classification and histology was accepted as a reference standard method.

**Results and discussion:** Normal results after cervix examination with scanner TruScreen were found in 82 patients (80,3%) and abnormal results were found in 20 patients (19,6%).

Squamous intraepithelial lesions detected with PAP test were found in 15 patients (14,7%) among them LSIL in 11 (73%) and HSIL in 4 (27%).

Cervix biopsy was performed in 24 patients with squamous intraepithelial lesions and abnormal colposcopic results; histological examination confirmed LSIL in 15 patients, HSIL in 8 patients and one case of CA (carcinoma in situ).

Histologically confirmed results correlated in 83% with TruScreen results and results after conventional PAP test correlated only in 63%.

**Conclusions:** Real-time optoelectronic scanner TruScreen demonstrated good efficacy in detection of cervical squamous intraepithelial lesions in comparison with traditional diagnostic methods.
AGC – RETROSPECTIVE STUDY OF THE LAST 9 YEARS
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Objectives: Atypical glandular cells (AGC) identified by cytology, indicate the presence of atypical glandular cells that most commonly originate from the endocervix or the endometrium. AGC is associated with a premalignant or malignant lesion in 10 to 40 percent of cases. The objectives of this work were to determine the incidence of AGC in the UCL population and evaluate the diagnostic methods and surveillance of these situations.

Methods: Retrospective review of 73 cases referred to our Department with the cytologic diagnosis of AGC in the last 9 years (2002-2010).

Age, parity, contraceptive method, menopausal condition, smoking and symptoms were evaluated. 73 women have done colposcopy, 61 HPV typing, 73 pelvic ultrasound and 38 Hysteroscopy with endometrial biopsy (28 without lesion, 6 with endometrial polyp, 1 submucous myoma and 1 with hyperplasia). The average age was 43 (22-84), 16 were asymptomatic and 17 postmenopausal women.

42% of women with AGC had epithelial lesion (15 LSIL, 8 HSIL, 2 Endocervical Polyps, 1 Endometrial Polyp, 1 invasive Carcinoma and 1 in situ Adenocarcinoma). In women with AGC + ASCUS (6), 50% had lesion (1 LSIL and 2 HSIL). HPV typing was done in 61 cases with 17 positives.

Conclusions: 47% (34/73) of patients had some kind of lesion, most of them were squamous-cell lesions. Serious injuries occurred in women over 35 years. The immediate colposcopy for all patients and Hysteroscopy on those with more than 35 years seems to be the best approach for a diagnosis of an AGC cytology.

The presence of AGC on cervical cytology is a significant marker for neoplasia of the endometrium, as well as the squamous and glandular epithelium of the cervix. No single test is sensitive for detecting neoplasia at all of these sites. For this reason, initial evaluation of all women (including adolescents) with AGC or AIS includes a combination of testing modalities.

VULVAR INTRAEPITHELIAL NEOPLASIA – A DIAGNOSTIC CHALLENGE

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Objectives: Growing interest in vulvar intraepithelial neoplasia (VIN) as a premalignant condition of the vulva is because of its known connection to human papillomavirus (HPV) infection. Although the progression of VIN to invasive planocellular cancer is rare, prompt diagnosis and treatment may lead to the best possible quality of life. Diagnosing VIN in young women with systemic lupus erythematosus (SLE) who are negative for high-risk HPV subtypes is particularly challenging. In immunologically affected individuals, the molecular milieu is altered such that diseases that are not characteristic of the subject’s age may appear (1).

Methods: A female in her late 20s had a history of constant itching of the vulva for more than one year before she consulted a gynecologist. Because of her young age and she was diagnosed to have SLE years ago, her complaints were not taken seriously. The symptoms did not improve after the patient used corticosteroid cream. The gynecologist assumed she had vulvar disease, and performed complete diagnostic procedures, including physical, speculum, pelvic and ultrasonic examinations, vulvar and cervical cytology, microbiological analyses and HPV test for high-risk HPV subtypes from the uterine cervix and vulvar samples, and by using colposcopy, vulvoscopy, Collins test, and biopsy the diagnostic procedures were completed (2).

Conclusions: Proper diagnostic procedures are needed because women of any age are equally susceptible to vulvar disease. Currently, the most commonly suggested VIN terminology established by the International Society for the Study of Vulvovaginal Disease (ISSVD) involves malignancy potential, presenting age, and connection to HPV infections (3, 4). Women with usual type, uVIN, are often found to have cervical and/or vaginal intraepithelial neoplasia, and both conditions are associated with HPV infection. Differentiated, dVIN, a common illness among women in their 70s, is rarely connected with HPV infection, but it has greater malignancy potential. As vaccines against HPV have been in use for the last 5 years, the final conclusion concerning prevention of vulvar cancer is not yet clear, but is promising.

References:
A PILOT STUDY TO INVESTIGATE THE TREATMENT OF CERVICAL HUMAN PAPILLOMAVIRUS INFECTION WITH ZINC-CITRATE COMPOUND (CIZAR®)

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Objective: In the present study the potential therapeutic effects of zinc-citrate compound (CIZAR®) in women infected with high-risk human papillomavirus (HR-HPV) was investigated.

Methods: A total of 194 women diagnosed with HR-HPV infection using the Hybrid capture (HC) II assay with no evidence of high grade squamous intraepithelial lesions (HSIL) or worse by pap smear and colposcopy were enrolled. Among them, 76 women were treated by twice weekly self administered intra-vaginal infusion of 0.5mM zinc citrate solution containing CIZAR® for 12 weeks and were evaluated for clearance of the HR-HPV infection compared to 118 women without treatment (Control group).

Results: The 12 weeks zinc citrate solution treatment resulted in the elimination of HR-HPV in 49/76 (64.47%) patients compared to the spontaneous clearance of 15.25% (18/118) in the control group (p=0.000). By logistic regression analysis, the 12 week zinc citrate solution treatment reduced the risk of persistent HR-HPV infection significantly (OR 0.079; 95% CI 0.039-0.165; p=0.000).

Conclusion: The results of this study showed for the first time that treatment with intra-vaginal infusion of a zinc-citrate compound (CIZAR®) can result in elimination of HR-HPV infection from the uterine cervix.

CERVICAL CANCER IN YOUNG FEMALE.

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Introduction: Portugal has a high incidence and mortality rate for cervical cancer, accounting for about 3-4% of deaths due to malignant tumours in the south region. Cervical carcinoma under 30 years of age represents less than 5% of all tumours diagnosed. Although it is rare in this age group, these females have the biggest impact for the reproductive future.

Objective: To investigate the diagnostic and prognostic aspects of cervix carcinoma in women aged 30 and younger.

Patients and methods: We retrospectively studied 76 patients, age equal or less than 30 years old, who were being treated for invasive cervical carcinoma at IPO, Lisbon, Portugal, from January 1995 to May 2006. Demographic and clinical data were analysed.

Results: Mean age was 28 years and mean time between first sexual intercourse and disease diagnosis was 10 years. Most of women (80%) used oral contraception and 23% were nulliparas. Only 4% of them had immune system compromise and 60% smoked. Almost all (90%) presented with syndromes (vaginal haemorrhage, pain or vaginal discharge). Disease stage was IA1: 1(1,3%), IB1: 18 (23,7%), IB2: 17 (22,4%) II A: 10 (13,2%), II B: 16 (21,1%), III B 12: (15,8%) IV A: 1 (1,3%) and IV B :1 (1,3%). Histopathologic classes included 59 (77,6%) squamous cell carcinomas, 16 (21,1%) adenocarcinomas and 1 (1,3%) neuroendocrine . Treatment used was chemotherapy (26), combined irradiation and hysterectomy (19), irradiation alone (15) and radical hysterectomy (7). Three patients were treated with traquelectomy in France. Ten patients had recurrent disease and seven developed metastasis. Five-year survival rate was 87% for stage I, 20% for stage II, 8% for stage III and 0% for stage IV.

Conclusion: Cervical carcinoma in young age has poor prognosis. As in Portugal most of the screening is opportunistic the new vaccines have launched a hope in reducing the incidence and mortality of this carcinoma.
STUDY ON THE CLINICAL STRATEGY OF CERVICAL LESION IN GESTATION PERIOD

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Objectives: To study the clinical strategy of cervical lesion in Gestation period

Methods: There are 31 cases of pregnant women attended the colposcopy clinic in our hospital. Among them, there are 28 cases with abnormal cytological results, 16 cases with ASC-US- 4 cases with LSIL, 8 cases with HSIL, respectively. The rest patients are 3 pregnant women with vaginal abnormal bleeding companied with normal cytological results. The patients age is from 21 to 36 years old, the average age is 29.4 years old. They all have colposcopy detection. If some cases were suspected with CIN, biopsy will be conducted. If the pathology result is or more serious than LSIL, colposcopy will be conducted to check the lesion change every 8 to 12 weeks until 36 weeks. The result is as followed. In these 31 cases, no complication was occurred induced by colposcopy detection. Biopsy was given in 18 cases. - High risk hpv detection are all negative in the 3 cases with normal cytological result. The bleeding reson are cast decidu for 2 cases, the other one is cervical poly. - For 16 cases with ASC-US, the pathology are 3 case with CIN1, 1 case with CIN3. The 4 cases with CIN are all postive for HR-HPV. The other 3 case is negative. The other 9 cases didn’t take any biopsy. Among them, 3 cases is HR-HPV positive, the two of them is subclinic papilomavirus infection, the other one is cast decidu. For 6 cases with HR-HPV negative, no any abnormal image was found in colposcopy detection. - For 4 cases with LSIL, 3 of them is HR-HPV positive, the pathological results is 1 with CIN2, 1 with CIN1, 1 negative. The other one is HR-HPV negative, the image in colposcopy is subclinical Papilomavirus infection. After caesarean section, the patient with CIN2 was checked and taken biopsy again, the pathological result is CIN3. CKC was followed, and the pathological result is CIN2. - 8 cases with HSIL result, their HR-HPV are all postive. Images in colposcopy are all implied HSIL. Pathological results is 7 cases with CIN3, 1 with CIN2?one of CIN3 is ended in early pregnancy, CKC was followed and pathological result is CIN3. One is still on going. The other 5 cases with CIN3 was check during pregnancy. No significant progress was found, so no biopsy is taken. After delivery, they are checked again, 3 cases with CIN1(2 cases vaginal delivery, 1 caesarean section), 2 with CIN3(1caesarean section), 1 with CIN3(1caesarean section). These 2 case of HSIL are conducted with CKC, pathological result are all CIN2. 1 case is missed.

Conclusion: HR-HPV testing is a useful strategy for pregnant women with abnormal cytological result. If cervical cancer is excluded, HSIL in pregnancy can be waited till delivery.

LOOP EXCISION FOR HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION ON CYTOLOGY: CORRELATION WITH HISTOLOGIC FINDINGS

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Objective: Our purpose was to examine the correlation between colposcopic and histologic findings in patients who have undergone loop electrosurgical excision of the cervix (LEEP) for high-grade squamous intraepithelial lesion (HSIL) on cytology without prior colposcopically directed biopsy.

Methods: A retrospective review was performed of all patients who underwent LEEP for high-grade squamous intraepithelial lesion (HGSIL) on Papanicolaou (Pap) smear without a prior cervical biopsy over a 50-month period. We correlated the histologic result at the time of LEEP.

Conclusions: Of 117 patients undergoing LEEP, 69 patients (67.5%) had cervical intraepithelial neoplasia (CIN) grade 2 or greater by histology and 28 patients (23.9%) had no pathologic findings, cervicitis or atypia. Six patients (5.2%) had carcinoma, 1 (0.9%) in situ adenocarcinoma, 2 (1.7%) invasive squamous cell carcinoma, 2 (1.7%) microinvasive squamous cell carcinoma and 1 (0.9%) microinvasive squamous cell carcinoma plus in situ adenocarcinoma. In all patients with carcinomas was realized hysterectomy. HSIL on Pap smear without prior colposcopically directed biopsy is an appropriate indication for LEEP.

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DIFFERENCE OF CLINICAL BACKGROUND BY HPV GENOTYPES AMONG THE PATIENTS UNDERGONE WITH THERAPEUTIC CONIZATION

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Objectives: We analyzed the difference of clinical background between HPV16 and HPV52/58 which are high detection frequency and also have high carcinogenic risk among the patients undergone with therapeutic conization.

Materials and Methods: Between Jan. 2008 to Dec. 2010, two hundred seven cases were performed with therapeutic conization (age distribution: 20 y.o. -73 y.o.), who diagnosed as CIN3 by punch biopsy in our institute. Patients showed single or mixed HPV infection by the methods of multiplex PCR preoperatively.

Results: HPV genotypes among the patients undergone with therapeutic conization were most frequently type 16 in 101 (48.8%) cases and 83% in total with 70 cases with HPV52/58. Age distribution was median 32 years-old in HPV16 including 23 cases of 32 years-old and almost half 52 cases from 30 to 34 years-old, but median 38.5 years-old in HPV 52/58 including 20 cases of 40 years-old. This showed statistically significant difference (p<0.001). Patients infected with HPV 16 were younger than those with HPV 52/58 at the timing of therapeutic conization. Not married rate and not parous rate in those patients with HPV 16 were 65% and 72%, respectively, but 48% and 42% in those with HPV 52/58, respectively. Preoperative cervical lesions in those with HPV16 were distributed in entire cervix among 72% of those, but much smaller lesion in those with HPV 52/58 (p<0.001). Above these, those with HPV16 might be more important about the preservation of fertility. And also those with HPV16 showed broader cervical lesions might be necessary with wider excision at conization, followed by more frequent infertility postoperatively.

Conclusion: Patients infected with HPV16 might be treated at the timing of diagnosis with not only CIN3, but also CIN2 with earlier decision and at smaller lesion for therapeutic conization.

AN 18 YEAR OLD GIRL WITH A CLEAR CELL ADENOCARCINOMA OF THE CERVIX: A CASE REPORT

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Introduction: Squamous cell carcinomas account for approximately 70 percent of cervical cancers, adenocarcinomas 25 percent, and adenosquamous carcinomas 3 to 5 percent. In addition, clear cell adenocarcinoma (CCA) of the uterine cervix is rare. CCA has been most commonly seen in women with prior intrauterine diethylstilbestrol (DES) exposure history.

Case Report: We report a case of an 18 year old nulliparous girl to whom a Clear Cell Adenocarcinoma of the Cervix (FIGO - stage IIa2) was diagnosed, with no previous history of DES exposure. On May 2010, she visited a physician with a 2 month history of irregular vaginal bleeding. Her menarche was at the age of 14 and the first intercourse at 16. She had no relevant past medical history. The gynaecologic exam showed a bleeding tumor, of approximately 5cm in size, protruding from the cervical os and filling the vagina. The histological diagnosis by punch biopsy was a CCA of the uterine cervix. After diagnosis, she was submitted to a Radical Abdominal Hysterectomy and Pelvic Lymphadenectomy, on May 2010. Pathological diagnosis was a greater than 4 cm CCA of the cervix, with invasion of the upper third of the vaginal, without parametrial invasion. No lymph node metastases were found. Following surgery, she underwent adjuvant vaginal brachytherapy. Reassessment 6 months after treatment showed no evidence of residual/recurrent disease.

Discussion: Although DES has not been prescribed to pregnant women for the last 30 years, CCA is rare but it is still relevant in our times. A Netherland 73 CCA cases review suggests that menarche and menopause may play a role in promoting carcinogenesis of women without intrauterine DES exposure. However information on the clinical behavior, pathology, and prognosis of these tumours is sparse and inconsistent, due to their rarity.
Objective: To assess the health and economic impact of a quadrivalent (6,11,16,18) HPV vaccine on the burden of genital warts (GW) in the UK.

Methods: A published mathematical model of the transmission dynamics of HPV infection and associated diseases was used to assess the potential impact on GW conferred by a quadrivalent HPV vaccine. Updated model inputs for GW costs were obtained from a recent cost study in the UK. We compared current cervical cancer screening practices combined with routine vaccination of girls aged 12, with a coverage rate of 80%, to screening without vaccination.

Results: The model predicted that vaccination versus no vaccination would reduce the number of HPV16/18-related cases of cervical cancer and cervical precancerous lesions (CIN2/3) by 86% and 85% respectively in the UK at year 100. In addition, quadrivalent HPV vaccination resulted in a reduction of HPV 6/11-related GW cases (in males and females) of 54,584 at steady state, corresponding to a reduction of 81%. The reduction in the incidence of GW was estimated at 54% in the sixth year of implementation of vaccination. The same trend was observed for GW associated management costs: 81% of GW costs were avoided at year 100. In the first 5 years of vaccination, 99% of the total HPV-related diseases cases and discounted costs avoided were attributable to the prevention of HPV6/11 related diseases. The cost-effectiveness ratio of a quadrivalent HPV vaccine was £5,254 per quality adjusted life year gained versus no vaccination. The cost-effectiveness ratio increased to £9,645 if vaccination was assumed to have no efficacy against GW.

Conclusions: A quadrivalent vaccine could help reduce the burden of GW in the UK, and thereby deliver important cost-offsets for the NHS, within an acceptable range of cost-effectiveness.

Objective: To determine the clinical significance of cervical cytologies with atypical glandular cells (AGC), by evaluating the performance of colposcopy, endocervical cytology (EC), endocervical curettage (ECC) and correlation with histopathology of LEEP/hysterectomy.

Methods: Revision of 50 women with a diagnosis of AGC at the Pathology and referred to Gynecology, between 2005 and 2010.

Conclusions: Mean age was 48.5 years [18-77]. Fifteen (30%) women were postmenopausal. Thirty-five (70%) were referred to colposcopy. The results were: no lesion, 19 (38%); suggesting LSIL, 11 (22%); suggesting HSIL, 5 (10%). EC was performed in 21 (42%) patients. The results were: normal, 12 (24%); LSIL, 4 (8%); HSIL, 1 (2%); AGC, 4 (8%). ECC was performed in 28 (56%) cases: normal, 14 (28%); insufficient, 6 (12%); LSIL, 2 (4%); ungradable dysplasia, 2 (4%); cervicitis, 1 (2%); carcinosarcoma, 1 (2%); squamous carcinoma in situ, 1 (2%) and adenocarcinoma in situ (AIS), 1 (2%). Nineteen (38%) women were submitted to transvaginal sonography (TVS) that was normal in 14 (28%), revealed an endometrial polyp in 2 (45%), hydrometra in 2 (4%) and thickened endometrium in 1 (2%). Hysteroscopy was performed in 6 (12%) women: normal, 3 (6%); endometrial polyp, 3 (6%). The histopathological results of the 25 (50%) women submitted to an excisional procedure were: normal, 10 (20%); CIN 1, 6 (12%); CIN 2, 3 (6%); CIN 3, 3 (6%); carcinosarcoma, 1 (2%); AIS, 1 (2%) and invasive adenocarcinoma, 1 (2%). Considering the performance of colposcopy in the detection of cervical pathology, it had a positive predictive value (PPV) of 0.86; a negative predictive value (NPV) of 0.62, a sensitivity (Se) of 0.67, a specificity (Sp) of 0.83 and an accuracy (Ac) of 0.73. Regarding EC, the results were: PPV=0.5; NPV=1; Se=1; Sp=0.5; Ac=0.67. For ECC, PPV=0.67; NPV=0.8; Se=0.8; Sp=0.67; Ac=0.73. In total, there were 15 (30%) cases of underlying preinvasive or invasive lesions among the studied patients (invasive adenocarcinoma, carcinosarcoma, AIS, CIN 1, 2 and 3). EC was more sensitive but less specific than ECC. The accuracy of colposcopy was similar to the ECC and higher than EC. In conclusion, the diagnosis of AGC in our population is clinically significant, due to the high prevalence of underlying preinvasive or invasive disease (30%).
HPV-18 IS A POOR PROGNOSTIC FACTOR, UNLIKE THE HPV VIRAL LOAD, IN PATIENTS WITH STAGE IB-IIA CERVICAL CANCER UNDERGOING RADICAL HYSTERECTOMY

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Objectives: This study was conducted to determine the prognostic significance of the human papillomavirus (HPV) genotype using the HPV DNA chip (HDC) test and the HPV viral load by the hybrid capture II assay (HC2) in FIGO stage IB-IIA cervical cancer undergoing radical hysterectomy.

Methods: Between January 2001 and December 2005, 204 consecutive patients who underwent radical hysterectomy with pelvic lymphadenectomy for International Federation of Gynecology and Obstetrics (FIGO) stage IB1-IIA cervical cancer were retrospectively reviewed. The Cox proportional hazards models adjusted for covariates were used for analyses and a receiver operating characteristic (ROC) curve was used to determine the HPV viral load in predicting disease progression. Of the 204 cases, the HDC was positive in 195 (95.6%) and the HC2 was positive in 192 (94.1%). The 5-year progression-free survival (PFS) was 78.4%. On multivariate analysis, HPV-18 positivity was an independent prognostic factor predictive for disease progression. The risk of recurrence was higher for HPV-18 positivity (hazards ratio = 2.664; 95% confidence interval [CI], 1.437-4.938; P = 0.003). The 5-year PFS rate for patients who were HPV-18-negative was 83.8%, which was higher than the 5-year PFS for patients who were HPV-18-positive (54.1%; P < 0.001). The area under the ROC curve for the HPV viral load was 0.550 (P = 0.314; 95% CI, 0.455-0.644).

Conclusions: The HPV-18 genotype is a reliable prognostic factor of early-stage cervical cancer; however, the HPV viral load may not be helpful in predicting disease prognosis.

THE SIGNIFICANCE OF HPV GENOTYPE IN PATIENTS WITH EARLY CERVICAL CANCER WHO UNDERGOING RADICAL HYSTERECTOMY

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Objectives: The aim of this study was to evaluate the prognostic significance of HPV genotype and the association of HPV genotype with other clinicopathologic risk factors in patients with early-stage cervical cancer who undergoing radical hysterectomy (RH).

Methods: A total of 173 patients with early-stage cervical cancer were included in this prospective study. HPV genotyping was performed on the cervical smear using PCR-based DNA chip test for 21 high-risk HPV types. Patients were divided into three groups according to the HPV genotypes; HPV 16 group, HPV 18 group, and non-HPV 16 & 18 group.

Results: Eighty-nine patients (51.4 %) had HPV 16 DNA, 28 (16.2 %) had HPV 18 DNA, 44 (22.5 %) had other types of high risk HPV DNA, and 12 (6.9 %) were negative for high-risk HPV DNA. Last two groups were regarded as non-HPV 16 & 18 group. There were no significant differences in menopause, parity, the International Federation of Obstetrics and Gynecology (FIGO) stage, histology of tumor, tumor size, depth of cervical stromal invasion, lymphovascular space invasion, resection margin involvement, parametrial involvement, and lymph node metastasis among the three groups. However, patients of HPV 16 group or HPV 18 group were significantly younger than those of non-HPV 16 & 18 group (, 46.1 years, 45 years and 51.4 year, respectively, P = 0.013). HPV 18-containing cancers were more likely to be adenocarcinomas compared to HPV 16 group or non-HPV 16 & 18 group (64.3%, 23.6%, and 16.1 %, respectively, P < 0.001). The proportion of patients who received adjuvant therapy and the type of adjuvant therapy were not different among three groups. HPV 18 group was associated with significantly higher rate of recurrence compared to HPV 16 groups or non-HPV 16 & 18 group (17.9 %, 2.3 %, and 3.6 %, respectively, P = 0.004).

Conclusions: Of early-stage cervical cancers, HPV 18-containing cancers were more frequently associated with younger patients with adenocarcinoma and was more likely to relapse after RH.
THE ASSOCIATION BETWEEN HPV VIRAL LOAD AND CLINICOPATHOLOGIC RISK FACTORS IN EARLY-STAGE CERVICAL CANCER

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Objectives: Clinical significance of pretreatment high-risk HPV viral load has been suggested in cervical intraepithelial neoplasia and advanced-stage cervical cancer. However, its role has not yet been clarified in early-stage cervical cancer. The aim of this study was to evaluate the relationship between pretreatment high-risk HPV viral load and clinicopathologic risk factors and prognosis of early-stage cervical cancer.

Methods: A total of 210 patients with early-stage cervical cancer who underwent radical hysterectomy were included in this study. HPV viral load was semiquantitatively measured in the cervical smears using a commercially available second-generation hybrid capture microplate-based HPV test. Relative light unit/cutoff (RLU/CO) ratios were calculated as the ratio of the specimen luminescence to the luminescence of the 1.0 pg/mL HPV 16 cutoff standard; RLU/CO ratios 1.0 were considered positive. HPV viral load was divided as high or low according to median HPV viral load and examined for its clinical significance.

Results: There were no significant differences in age, parity, menopause, body mass index, the International Federation of Obstetrics and Gynecology (FIGO) stage, tumor size, depth of cervical stromal invasion, lymphovascular space invasion, vaginal involvement, and resection margin involvement between high and low viral load groups. However, patients with low viral load was more likely to have adenocarcinomas (22.2 % vs. 16.3 %, P = 0.057). High viral load was significantly associated with parametrial involvement (8.6 % vs. 18.1 %, P = 0.042) and lymph node metastasis (7.6 % vs. 17.1 %, P = 0.036).

Conclusions: Our study was the first one which suggested the relationship between pretreatment high-risk HPV viral load and clinicopathologic risk factors in early-stage cervical cancer. Further evaluation is required to clarify the prognostic role of pretreatment high-risk HPV viral load.

RECONISATION OR REPEATED HPV TEST?

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Objective: Is the second (pre-reconisation) HPV test an appropriate method to reduce the number of interventions in histologically positive cases?

Study design: 438 cervical conisations - loop electrosurgical excision procedures (LEEP) - were performed between March 2008 and August 2010 at our Dept. of Gynecology. Samples for high-risk HPV test (Genoid, Hungary) were taken from the surface of the cervix and from the cervical canal before the LEEP procedure and histopathological examinations were performed. Margin positivity was the indication for reconisation (re-LEEP).

Results: 119 (27.2 %) out of 438 cases were reconisations. In cases of histologically proven residual dysplasia (29 of 119) high-risk HPV infection was detected by HPV test, too. In ninty cases (90 of 119) residual dysplasia was not seen by histological examination. In this group high-risk HPV infection was not detected in 77 cases (85.5%) by the time the second HPV test was performed. HPV tests for high-risk types were positive only in 13 of 90 (14.5%) without residual dysplasia. Furthermore the same HPV type was detected only in 3 cases taken before the first and the re-conisation procedure.

Conclusion: Pre-reconisation HPV testing might be useful in reducing the number of reconisations where high-risk HPV test is either negative or does not confirm the previously proven HPV type.
HPV PROPHYLACTIC VACCINE COVERAGE IN FRANCE: RESULTS OF A SURVEY AMONG HIGH SCHOOL AND UNIVERSITY STUDENTS IN MARSEILLES' AREA

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Objective: To assess HPV prophylactic vaccine coverage among French high school and university students as well as their level of education about this vaccine.

Materials and Methods: An anonymous survey was conducted among 2500 high school and university students from the area of Marseille, France, from December 2009 to April 2010.

Results: A total of 2018 questionnaires were collected (80.7% participation rate). Only 671 (35.4%) participants reported having been vaccinated against HPV, of whom 510 (73.4%) had completed the 3 injections scheme. Among those not being vaccinated, 671 (49.8%) fulfilled criteria for a catch-up vaccination, of whom only 325 (48.4%) agreed for such a catch-up. Main reasons given for refusal for a catch-up vaccination were the lack of information about HPV vaccine and fear of side effects. Practice of cytological cervical cancer screening was not significantly influenced by vaccination status. Thus, 578 (45.2%) participants who had not been vaccinated already had had a cervical cytology performed, versus 295 (43.3%) vaccinated ones (p = 0.445). In total, 1722 (90%) considered themselves as educated about the HPV vaccine. Source of education was attributed to doctors and media by 54.4% and 53.7% of participants, respectively. Educational role attributed to school and university was poor (3.4%).

Conclusion: Despite apparent satisfactory level of education, HPV prophylactic vaccine coverage among high school and university students appears to be insufficient.

REASONS FOR HPV VACCINE NON-COMPLIANCE IN WOMEN AGED BETWEEN 18 TO 26 YEARS OLD, ACCORDING TO AN HPV VACCINATION RECORDING NETWORK IN ATHENS

Deligeoroglou E1, Salakos N1, Bakalianou K1, Georgandopoulou A2, Papachristoforou C2, Papanedorou D4, Pappas A5, Diakakis I6, Solidakis A3, Papadimitriou A7, Kasanos D5, Creatsas G1
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3 Maternity Hospital «MITERA» Athens - 4 Department of Ob-Gyn , Metaxa Cancer Hospital Of Piraeus
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6 3d Obstetrics Gynecology Clinic of Public Hospital «Manika iliadis» - 7 Department of Ob-Gyn, General Hospital ,Nikaia «St.Panteleimon»

Objectives: The aim of this study is to assess the issues surrounding HPV vaccine implementation, among women 18-26 years old.

Methods: An HPV Vaccination Recording Network, under the auspices of the 2nd Department of Ob-Gyn at the University of Athens, in “Aretaieion” hospital is being established, to collect data in a uniform database, in collaboration with 6 major participating public Gynecological Clinics in Athens, from women who had been referred for screening and/or HPV vaccination. A total of 183 young women aged between 18 to 26 years old, during the last six months, were recorded and were studied, in order to examine the HPV vaccine implementation, in those women who were screened and in parallel were informed about HPV vaccination. Among a sample of 100% who were screened, only 15% of them had been vaccinated with at least one shot of the HPV vaccine, and only 8% of them had been vaccinated with multiple vaccine shots. More than 50% had multiple risk factors for HPV infection. Awareness of HPV or the presence of >2 risk factors, were both associated with increased perceived risk, but neither perceived risk status nor the number of risk factors predicted willingness to HPV vaccine implementation. The past medical history of HPV related diseases and Genital Warts increased HPV vaccine implementation. Frequent causes responsible for low HPV vaccination rates include: monogamous sexual relationship, incorrect information about the benefits of HPV vaccination, past medical history of HPV infection in young women, low perceived risk for HPV infection, concerns about vaccine side effects.

Conclusions: These results demonstrate that the presence of multiple risk factors for HPV infection does not increase women’s willingness to HPV vaccine implementation. This highlights the need for further patients education, especially in those young women who have already been infected with HPV.
FACTORs ASSOCIATED WITH ATTENDANCE TO CERVICAL SCREENING IN AN ORGANIZED SCREENING PROGRAM

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Objectives: To address the predictors of compliance to an organized cervical screening program by comparing characteristics of spontaneous attendees, reminded attendees and nonattendees to cervical screening in Norway.

Methods: A questionnaire on lifestyle and health was administered to a random sample of women, of which 68% responded. A total of 12,058 Norwegian women aged 25-45 were eligible for the present study. Their questionnaire data was linked to the databases of the Norwegian Co-ordinated Cervical Cancer Screening Program (NCCSP) to establish each woman’s screening attendance. We distinguished between nonattendees, spontaneous attendees and reminded attendees to screening during the past four years. Predictors of nonattendance versus attendance and reminded versus spontaneous attendance were established by multivariate logistic regression.

Conclusions: Nonattendance to cervical screening was associated with no history of sexually transmitted infections, never-use of hormonal contraceptives, not having given birth, current smoking and not having an opinion about the recommended screening interval. Single status, unawareness of the recommended screening interval, never-use of condoms and infrequent drinking of wine were associated with a less pronounced increased likelihood of nonattendance. Among attendees, women who were older, who never had a sexually transmitted infection or who were aware of the recommended screening interval were more likely to be reminded rather than spontaneous attendees. Educational level did not significantly affect the women’s attendance status. We conclude that awareness of cervical screening as well as gynecological events that involved consulting a physician strongly influenced attendance versus nonattendance and reminded versus spontaneous attendance in an organized screening setting. Our results indicate that an increased awareness about cervical screening in the population may increase attendance. Moreover, the relatively low awareness of spontaneously screened women suggests a need for improved information from physicians when smears are taken at the physician’s initiative.

EVALUATION OF HPV VACCINATION COMPLIANCE ACROSS DIFFERENT AGE-GROUPS FROM A HPV VACCINATION RECORDING NETWORK IN ATHENS

Drakakis P1, Tsetsa P1, Sotiropoulou M1, Michala S1, Katsoulis M2, Panotopoulou E3, Tessas H4, Patrikios G3, Roslymous C6, Tserkezoglou A7, Antsaklis A1

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3 Department of Virology, Papanicolaou Research Centre of Oncology and Experimental Surgery
4: Department of Ob-Gyn, of 251- Airforce General Hospital
5: Department of Ob-Gyn, of 401- General Army Hospital
6 4th Department of Ob-Gyn of Public Hospital “Marika Iliadi”, Athens
7 2nd Department of Gynecology, Regional Anticancer Oncology Hospital of Athens “St. Savvas”, Athens, Greece

Objectives: The aim of the study is to examine the presence of disparities in HPV vaccination compliance on different age-groups and to examine the relation with pre-specified factors, based on a HPV Vaccination Recording Network under the responsibility of the 1st Gynecological clinic of University of Athens collecting data in uniform database from 6 major participating public Gynecological clinics of Athens. Although the size of the sample is enable to exact accurate data about the National vaccination coverage, it can be estimated regarding the vaccination compliance.

Methods: In total 350 girls and young women recorded by the Vaccination Network were studied and stratified in 4 different age groups. Group A: 12-15 yrs old, Group B 15-18 yrs old, Group C 19-26 yrs old, Group D:27-45 yrs old.Regarding vaccination coverage, the highest vaccination coverage of 23% was in the age group of 18-26 yrs old, followed by descending order of the age group of 15-18yrs (15%), 12-15yrs (12%)and 27-45yrs(4%). The highest vaccination compliance 90%, was observed in the age group of 12-15 yrs old followed by the age group 26-45yrs (89%),18-26yrs(78%) and 15-18yrs (75%).

Conclusion: Regarding the above disparities in the HPV vaccination compliance among different age groups highlights the best compliance in primary cohort and highlights the necessity of improved scientific patients consultation from the physician, thus decreasing the effect from non-scientific factors to the decision of vaccination.
In Croatia, invasive cervical cancer (ICC) continues to be the eighth most common malignancy in women. In 2007, there were 387 newly detected ICC cases and 114 related deaths (age-standardised rates for incidence -11.5/100 000 and mortality - 3.0/100 000). Although the opportunistic screening by Pap smear has been conducted since 1950s, Croatia continues with unfavourable trends in ICC mortality compared to other European countries. A downward ICC incidence trend recorded between 1970 and 1991 has been stopped and reversed upwards. The highest incidence rates of ICC and CIN III are reported in age groups 40-59/80-84 and 30-34, respectively.

The Croatian National Program for Early Detection of Cervical Cancer planned to start in 2011, envisages Pap smear for women aged 25-64 once at 3 years, and also includes public education, targeted primarily at youth, about protection against sexually transmitted infections (STIs), especially HPV infections as the major cause of ICC.

In the meantime, during the European Cervical Cancer Prevention Week, the Croatian League Against Cancer regularly organizes Mimosa Day, using this fragile symbol of female solidarity to remind women all around Croatia of the importance of cervical cancer education, vaccination and regular screening, thus raising public awareness in this matter to achieve better protection against STI, higher vaccination coverage, and larger (at least 80%) response to gynaecological screening. The result is a 15% increase in gynaecologic visits in Zagreb during the first 3 months following the campaign, which certainly contributes to a reduction of cervical cancer in Croatia.
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**Posters Index**

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